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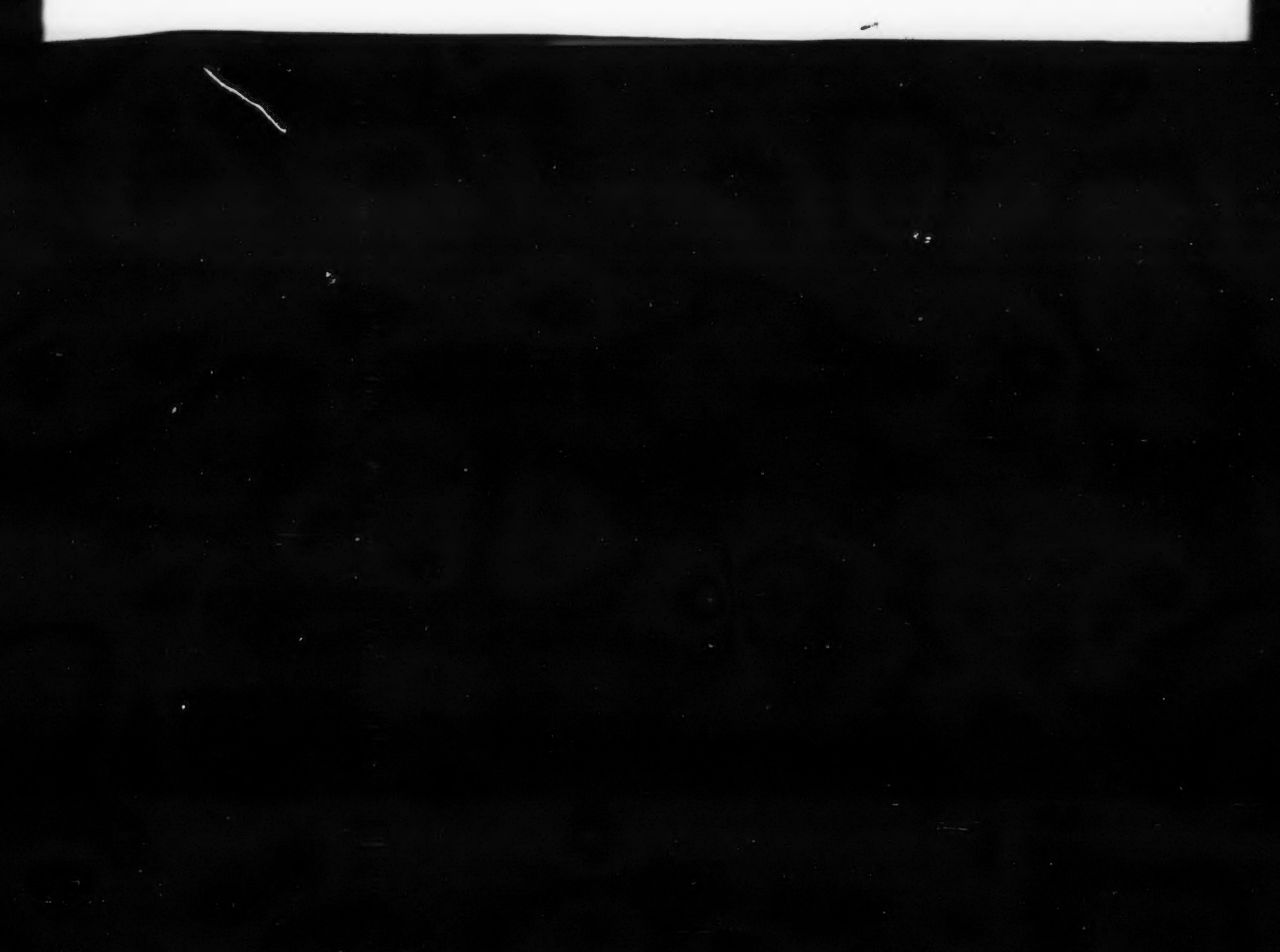
## ERRATUM

In the papers on Baths by Bazett and Bazett, Thurlow, Crowell and Stewart in the October, 1924, number of this JOURNAL, volume 70, pp. 412 and 430, all values given for acid and $NH_3$ excretion per hour should be milliequivalents multiplied by 10 and not milliequivalents, as stated.



THE AMERICAN
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# STUDIES ON THE INNERVATION OF SMOOTH MUSCLE

## I. Vagus Effects on the Lower End of the Esophagus, Cardia and Stomach of the Cat, and the Stomach and Lung of the Turtle in Relation to Wedensky Inhibition[1]

HARRY O. VEACH

*From the Laboratories of Physiology in the Harvard Medical School*

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In the course of researches carried out in 1908, Prof. W. B. Cannon observed incidentally that low frequencies and intensities of stimulation of the vagus nerve in the cat had a pronounced motor effect on the cardiac sphincter, whereas higher frequencies and intensities were inhibitory. Similar effects are produced in the same way in the nerve-muscle preparation of the frog when the sciatic nerve is stimulated, and the relaxation of the gastrocnemius, which occurs on increasing within limits the frequency and intensity of stimulation, is the well-known Wedensky inhibition. This similarity in the mode of production of corresponding effects in the two neuro-muscular mechanisms suggested to Professor Cannon that the inhibitory reactions in both might be of the same fundamental nature.

The importance of such observations is obvious when it is considered that physiologists have studied intensively the phenomenon of inhibition for more than three-quarters of a century, and though much has been added to our knowledge in regard to its manner of occurrence and the organs in which it takes place, little is definitely known as to the actual processes involved in the reaction. It was suggested to the author by Professor Cannon, therefore, that further experiments on variation in frequency and intensity of stimulation of nerves to smooth muscle should be undertaken, in order that additional data bearing on inhibition might

[1] This paper presents some of the results of work done in partial fulfilment of the requirements for the degree of Doctor of Medical Sciences. A preliminary report of these results was published in Science, 1924, lix, 260.

be obtained. It is the purpose of the present paper to give an account of experiments on the action of the vagus on the lower end of the esophagus, the cardia and the stomach of the cat, and the stomach and lung of the turtle, and to point out its close relation to Wedensky inhibition.

PREVIOUS OBSERVATIONS CONCERNING VAGUS ACTION ON THE STRUCTURES IN CONSIDERATION. In mammals, the action of the vagus on those parts of the alimentary canal concerned is both motor and inhibitory. Openchowski (1), (2), (3) showed that stimulation of the vagus evokes both contraction and relaxation of the cardia of rabbits and dogs. He reported also certain observations on the relation of the effect produced to the strength and frequency of stimulation (1), (3) which may be summarized as follows: *a*, single induction shocks did not cause contraction; *b*, strong shocks at a frequency of 3 per second produced weak contractions of the cardia after a latent period of $\frac{1}{2}$ to 2 seconds; *c*, frequencies from 12 to 30 per second caused with moderate intensity contraction and with low intensity relaxation; *d*, frequencies of 50 to 60 per second with moderate intensity led to slight relaxation. Langley (4) found that atropin favored dilatation of the cardia of the rabbit, and that strong after-contraction occurred on cessation of stimulation. Meltzer and Auer (5) also observed that inhibition of the cardia of the rabbit occurred during stimulation, and that it was followed by after-contraction. Stimulation of the central end of the vagus had much the same effect, and they obtained evidence that the inhibition produced thus was exercised peripherally. Cannon (6), (7) observed that bilateral vagotomy in the cat leads to temporary paralysis of the lower end of the esophagus, often accompanied by hypertonicity of the cardia. Carlson, Boyd and Pearcy (8) have found likewise that the vagus has both motor and inhibitory effects on the lower end of the esophagus and cardia of the cat, and on the cardia of the dog and the rabbit. Carlson and Litt (9) have reported more recently contraction of the cardia of a macacus monkey on stimulation of the vagus.

Most investigators have found that the predominant action of the vagi on the mammalian stomach is motor (6, pp. 192–202), but there is much evidence that they also cause relaxation of the gastric musculature. Wertheimer (10), Morat (11) and Auer (12) have obtained good evidence that the vagi may be the efferent pathway for inhibitory reflexes to the stomach. Cannon and Lieb (13) found, moreover, that the receptive relaxation of the stomach of the cat, accompanying deglutition, fails to occur if both vagi are cut. Of interest in this connection too is the observation of Carlson, Boyd and Pearcy (8) that an increase in gastric tonus, persisting at least for several hours, may occur on bilateral vagotomy in the cat. That stimulation of the peripheral end of the vagus may cause inhibition of the stomach has been demonstrated also. Langley (4) obtained relaxation thus in the cat and rabbit. May (14), who carried

out experiments on dogs, cats, rabbits and monkeys, reported inhibition at the beginning of a period of stimulation of the vagus, which was replaced by contraction, if the stimulation was prolonged. In nearly all of May's experiments, however, enough atropin had been given to render the cardiac branches of the vagi ineffective.

In regard to the stomach of the turtle, Carlson and Luckhardt (15) observed that the contraction evoked by tetanization of the vagus is limited to the beginning of stimulation, relaxation taking place thereafter. This observation was confirmed by Bercovitz and Rogers (16), (17), though they report in addition a temporary relaxation preceding the contraction. Low frequencies of stimulation in their experiments, however, caused only dilatation. For a review of the literature on the reactions of the musculature of the turtle's lung to vagus stimulation, the paper by Carlson and Luckhardt (15) should be consulted. It may suffice to state here that nearly all investigators have reported only contraction.

EXPERIMENTAL METHODS. *a. Electrical apparatus.* In the first experiments, an inductorium provided with an automatic interrupter was used. This coil, which will be designated as coil B, was very satisfactory for a limited range of frequencies. It became desirable, however, to use a wider range of frequencies, and for this purpose a rotary interrupter was employed. The interrupting cylinder was about 7 cm. in length and 7 cm. in diameter. The body of the cylinder was constructed of brass, and in its surface brass segments were set, insulated from the conducting part by a layer of mica about 1 mm. thick. These segments were arranged in three encircling rows, and they served to interrupt the primary current. The first row contained only one such segment; the second contained eight, and the third thirty-two. On the circumference of the cylinder, the non-conducting and the intervening conducting portions were about equal in length, except in the third row where the ratio of the former to the latter was about 3 mm. to 4 mm. The method of revolving this cylinder and of determining its rate of revolution, as well as the contact brushes (phosphor-bronze), have been described by Forbes (18). It was possible with this arrangement to obtain frequencies of interruption from $1\frac{1}{4}$ to 1000 per second. A change from one frequency to another, for a given position of the contact brushes, moreover, could be made in the course of 2 seconds by varying the pressure exerted against the carbon plates of the resistance in the armature circuit of the motor (18).

String galvanometer records of the galvanic current interrupted by this apparatus, when the cylinder was well polished, showed very good uniformity of contact. The shocks induced in the secondary coils were correspondingly uniform. A record of a series of make and break shocks from coil A is reproduced in figure 1. In case irregularities in contact developed, they were repeated regularly for each revolution of the cylinder, and they did not affect qualitatively the results to be described.

Fig. 1. String galvanometer record of make and break shocks from coil A. Rotary interrupter: 8 interruptions of primary current per revolution; 50 per cent closure. Time in 0.01 second intervals. String tension, 68 m. per amp. Magnification, 490.

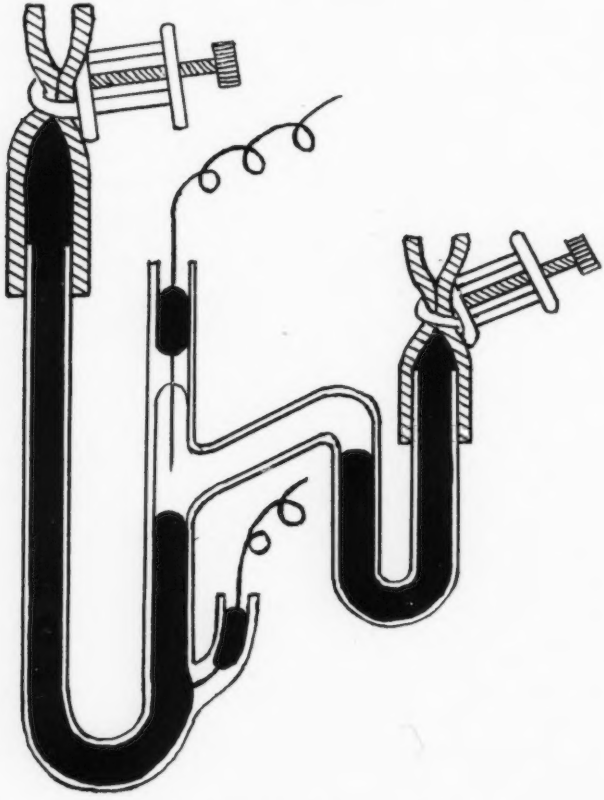

Fig. 2. Non-oxidizable mercury key

Inasmuch as the Martin knife-blade key develops mechanical defects (19), (20), (21), a nitrogen key was designed for the primary circuit which combines cleanness of the contact surfaces and durability. A diagram of its construction is given in figure 2. A glass tube bent in the manner

indicated has two platinum wires sealed into it, one near the top and the other at the bottom of its middle vertical portion. These communicate on the exterior of the tube with mercury cups, for connection with the poles of the current source. The upper wire is tapered to a point, and it is placed well in the center of the tube to insure contact with the uppermost part of the mercury meniscus when the current is made and broken. The unshaded part of the interior of the tube is filled with nitrogen at about 1.5 atmospheres pressure, and the parts represented in black are filled with clean mercury. The ends are closed with strong-walled rubber tubing. Contact is made by applying pressure to the longer of these tubes. Such a key remains clean for months, and string galvanometer records show that its action is very uniform.

A diagram of the arrangement of the electrical apparatus is given in figure 3. The combinations in which the various switches and keys were used in directing the primary current and in signalling the stimulation are indicated in the following schema, in which the letters, u and d, refer to the closure of the switches, whether upward or downward, and the letters, o and c, refer to the keys, whether open or closed.

1. Primary current through interrupter: 1d; 2u.
   a. Through coil A only: 3u; 6u; 9c.
   b. Through coil G only: 3d; 4u; 5d; 10c.
   c. Through coils G and A: 3d; 4u; 5u; 6u; 9c; 10c.
2. Primary current through mercury key: 1d; 2d.
   a. Through coil A only: 3u; 6d; 9o.
   b. Through coil G only: 3d; 4d; 5d; 10o.
   c. Through coils G and A: 3d; 4d; 5u; 6d; 10o; 9o.

Five induction coils were used in the course of the work. Coils A, G and B were used most extensively, and coils C and F only occasionally. Each was provided with an iron core in the primary. Coils A, F and G were constructed according to Kronecker specifications. Some of the details of construction are given in the table below.

| COIL | LENGTH OF SECONDARY | TURNS OF WIRE IN SECONDARY | RESISTANCE OF SECONDARY | MEAN DIAMETER OF SECONDARY | LENGTH OF PRIMARY |
|---|---|---|---|---|---|
| | *cm.* | | *ohms* | *cm.* | *cm.* |
| A | 12.5 | 10,000* | 860 | 5.5 | 14.0 |
| B | 6.0 | | 660 | 3.5 | 10.0 |
| C | 12.3 | | 2,370 | 4.5 | 14.0 |
| F | 13.0 | | 1,280 | 5.5 | 16.5 |
| G | 13.0 | 10,260* | 770 | 5.5 | 13.0 |

* From Martin, E. G. *The Measurement of Induction Shocks.* New York, 1912, p. 80.

All of the coils, with the exception of coil C, were calibrated by Martin's method (22). In calculating the physiological value of the shocks in

Z units, however, only the break shocks were considered, though the makes also affected the nerves. It was assumed that an increase in the intensity of the break shocks meant a roughly corresponding increase in

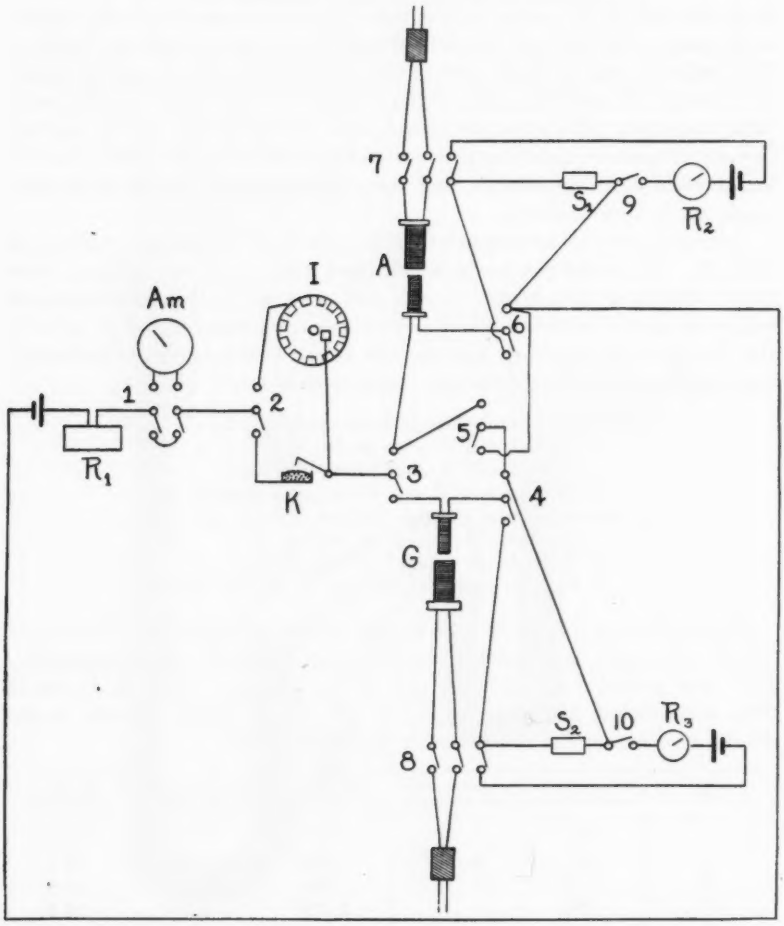


Fig. 3. Electrical apparatus used in stimulation. $R_1$ = variable resistance in primary circuit. *Am* = milliammeter properly shunted for measuring primary current. *I* = rotary interrupter. *K* = mercury key. *A* = coil A. *G* = coil G. Nos. *1*, *2*, *3*, *4*, *5* and *6* = copper switches in primary circuit. Nos. *7* and *8* = copper switches in secondary circuits of induction coils and in signal magnet circuits. $S_1$ and $S_2$ = signal magnets. $R_2$ and $R_3$ = variable resistances in signal magnet circuits. Nos. *9* and *10* = keys in signal magnet circuits.

the intensity of the makes (22). The strength of the shocks was varied by changing the position of the secondary coil with respect to the primary. No extra resistance other than that of the nerve trunk was included in the secondary circuits. When two inductoria were used simultaneously, they were placed at right angles to each other.

Neither the make nor the break shocks were short-circuited, inasmuch as Erlanger and Garrey (20) have shown that such a procedure may only prolong the process of induction, leaving the shock effective after the short circuit is broken. Hofmann (23) found, moreover, that the direction of the stimulating currents had little to do with the production of the Wedensky effect. Nor was any attempt made to equalize make and break shocks, for Hofmann (23) showed also that the ordinary inductorium produced the Wedensky effect as well as the electrical siren, the shocks from which were probably of equal intensity. In reckoning the frequency of stimulation, only the break shocks were counted. String galvanometer records showed that the duration of the make shocks from coils A and G was not greater than $5\sigma$, and that the breaks were not longer than $3\sigma$. The shocks from coil C, however, were probably twice as long. Records from coils B and F were not taken.

The primary current in most of the experiments was adjusted to 0.1 ampere, and it was delivered by a single 2 volt accumulator. The percentage closure for frequencies up to 120 interruptions per second was regularly 50 per cent. For higher frequencies, it was about 60 per cent. Some observations were made, in addition, with the brushes set to give 75 per cent closure. When the automatic interrupter of coil B was employed, however, different intensities of the primary current were used, and the duration of closure averaged probably 25 per cent.

Platinum wire electrodes, of about 0.7 mm. diameter, were used regularly, though variations in the composition of the electrodes did not affect the results. The wires were placed about 3 mm. apart, and the stimulated nerve trunk was drawn between them so that one was applied to the upper surface and the other to the lower surface in the manner described by Sherrington (24). In order to avoid electrotonic diminution of the effectiveness of the break shocks, the electrodes were so placed that the cathode for the breaks was peripheral to the anode when the peripheral end of the nerve was stimulated, and the reverse when the central end was stimulated. Sherrington glass shields (24) were employed regularly to prevent spread of current.

*b. Recording apparatus.* For recording volume and pressure changes in the viscera, the balloon and water manometer method was used exclusively. The cylinders of the manometers were cut from heavy walled glass tubing, and they varied in size corresponding to the magnitude of the movements to be recorded. For the stomach and lung of the turtle, they

were 7 mm. in diameter, an excursion of 13 mm. of their floats corresponding to a change in volume of the organ of 0.5 cc. For cats, the esophagus-cardia manometer was 11 mm. in diameter, an excursion of 1 cm. representing a volume change of 1 cc. in the corresponding balloon. The manometer for the stomach was 22 mm. in diameter, an excursion of 13 mm. corresponding to a volume change of 5 cc. Each cylinder was supplied with a celluloid cap with a hole bored in the center. At first the inner surfaces of the cylinders were coated with paraffin, as were also the floats, in order to prevent adherence of the latter to the walls. Later it was found that the paraffin coat of the floats alone was sufficient for this purpose. In each case, the floats were about 2 mm. less in diameter than the corresponding cylinders. Their weight was so adjusted that their upper surface stood about 2 mm. above the water level in the manometers. The larger floats were made of cork, and the smaller ones of pith. Mounted in the center of the float was a fine glass tube, which projected upward through the hole in the manometer cap, and was joined to a light horizontal glass writing tube, the point of which was fire polished. This horizontal writing arm traced the movements of the float on the smoked surface of the kymograph paper. In order to make the movement of the writing points of the larger manometers uniform, a third light tube was cemented at right angles to the two others, and this and the writing arm were made to move between silk guiding threads.

The balloons employed were made of delicate rubber. Their general shape when inflated is shown in figure 4. For the stomach of the turtle, the balloon was fixed by means of a rubber band to the end of a rather stiff rubber tube. The diameter of this tube on the exterior was about 4 mm., and on the interior about 2 mm. The pressure on the balloon averaged about 10 cm. of water. The lung of the turtle was connected with its manometer through air transmission, and it was kept inflated by about 1.5 cm. water pressure.

A diagram of the entire apparatus for recording the contractions of the lower end of the esophagus, the cardia and the body of the stomach of the cat is given in figure 4. A water reservoir of about 125 cc. capacity was connected with the stomach manometer, and a smaller reservoir with the esophagus-cardia manometer, to permit large excursions of the floats in response to pressure changes. The esophagus-cardia system was separated from that of the stomach by placing the transmitting tube of the latter inside that of the former. The outer tube was made of stiff rubber, so that contractions of the esophagus and pharynx were unable to compress it. These two tubes were connected to a specially designed enterometer (fig. 4, *E*). This device was constructed of brass. Its greatest outside diameter was 1 cm., and the lumen of its central tube was about 2 mm. in width. The end piece, 4 mm. thick, was deeply grooved. Two centi-

meters orad of this, an outer collar was fixed to the central tube. The balloons used were of sufficient length that they could be divided into two compartments by means of a rubber band applied at the groove in the end piece. Thus an esophagus-cardia balloon of 2 cm. length, fixed at its oral end to the brass collar, was separated from a stomach balloon of

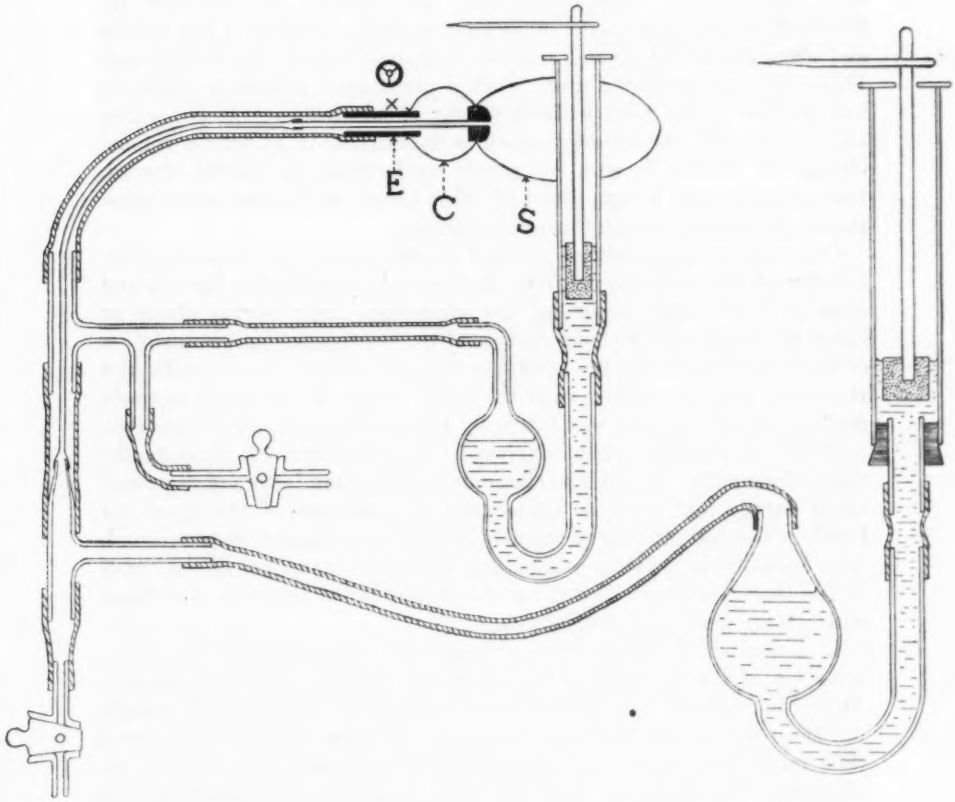


Fig. 4. Recording apparatus. $E$ = enterometer. $C$ = esophagus—cardia balloon. $S$ = stomach balloon.

about 9 cm. length. As figure 4 shows, air could be introduced readily into either system by way of stopcocks. The water pressure on the balloons averaged about 22 cm. In the earlier experiments on cats, contractions from an esophagus-cardia balloon only were registered, but the recording system was essentially the same.

*c. Preparation of animals.* A variety of methods was employed in the preparation of cats. Chloralose, urethane, ether, paraldehyde and sodium-barbital, in the order of frequency of use, were given as anesthetics. Chloralose became the drug of preference, for it left the alimentary canal in a state of moderate tonus and rhythmic activity, resembling that often found in the decerebrate preparation. In other experiments, the animals were decerebrated. Occasionally the decerebrator, as described by Sherrington (25), was used. A method involving ligation of the basilar and clamping of the carotid arteries, described recently by Pollock and Davis (26), was employed also. Such a preparation permits a relatively low position of the head without danger of hemorrhage. It is possible also to dissipate the rigidity, which is undesirable in recording volume changes of viscera in situ, by merely unclamping the carotid arteries. Decerebration was accomplished at other times, under deep ether anesthesia, by pithing through the optic foramina.

Other operative procedures included the insertion of a tracheal cannula, isolation of the vagus nerves from the cervical sympathetics, ligation and cutting of the vagi, sectioning the splanchnic major nerves above or below the diaphragm without rupture of the peritoneum, and cannulation of the femoral vein and the carotid or femoral artery. Occasionally also the spinal cord was pithed from the lower lumbar to the upper thoracic region. Blood pressure was taken by means of a mercury manometer. Artificial respiration, when employed, was administered through the tracheal cannula. In order to eliminate complicating factors of decreased blood supply and pressure consequent on inhibition of the heart, an excellent method introduced recently by Professor Cannon was employed. This consisted in isolating and cutting the cardiac branches of the vagus in the thorax, without disturbing the conduction of impulses over fibers with a more peripheral distribution.

In a number of experiments, arrangements were made for direct observation of the responses of the alimentary canal to vagus stimulation. An incision between two of the lower ribs in the left thoracic wall brought the lower end of the esophagus into view. Contractions could be traced thus until they disappeared beneath the diaphragm. For further observation, this structure was cut away from its attachments to the esophagus.

For observation of the reactions of the stomach, the entire animal was placed in a moist chamber. This method has a number of advantages over the saline bath introduced by Sanders (27) and used extensively by his collaborator, Houckgeest (28). It permits ready stimulation of deeply lying nerves without unnecessary spread of current, such as occurs in a saline medium; it leaves the viscera covered with peritoneal fluid, preventing contamination with urine and feces, and it obviates the labor and

expense of preparing large quantities of salt solution. Such a chamber was made by fitting a lid to a tank which formerly had been used for a saline bath. Three windows were cut into the lid: a middle large one to give a view of the viscera; a second smaller one over the pelvic region and lower extremities, and a third situated above the cervical region. These windows were covered with easily removable glass plates, which were slanted to prevent dripping of water on the exposed viscera. Fogging was prevented by rubbing on strong soap, this material being washed off in a stream of running water before the plates were placed in position. The lid also contained holes for artificial respiration tubes and a thermometer. The vapor was supplied by about 5 cm. of water, which covered the electric heating coil in the bottom of the tank. The temperature was kept at 37°C. by means of a thermoregulator.

The animal to be introduced was tied to a cat board of about 7 cm. height, and the necessary preparations were made for opening the abdomen in the median plane from the symphysis pubis to the xiphoid process. The electrodes were fixed in place also, and a mouth guard for admission of the balloons was applied. Transfer was made then to the moist chamber, and the balloons were inserted, having been introduced through a hole in the end of the box near the head of the cat. The wires leading to the electrodes, and the ligatures tied on either side of the linea alba were drawn out through holes in the sides of the box. Finally, after the temperature had risen to 37°C., the linea alba was cut through, and the margins of the abdominal wall were drawn apart for a view of the viscera. Under these conditions, with ordinary precautions to prevent excessive escape of moisture, the peritoneum keeps its lustre, the movements of the alimentary canal continue, and the excitability of the exposed nerves is retained, for hours.

The collapsed balloons were inserted usually through the mouth, but occasionally through an opening in the cervical portion of the esophagus. To prevent friction they were wet with Ringer solution, and the esophageal tube was coated with petrolatum. The position of the esophagus-cardia balloon with respect to the diaphragm, and therefore with respect to the cardia, could be ascertained by the respiratory variations in pressure in the manometer. Inspiration had a pressor effect if the balloon was below the diaphragm, and a depressor effect when it was above this structure. Further information as to the position of the balloon was obtained by the fall in pressure, which took place when it was pushed into the stomach, and the corresponding rise when it was pulled back into the esophagus. When the two balloons were employed together, little difficulty was experienced in keeping them in place. The larger balloon accurately filled the body of the stomach, extending from the cardiac orifice to the beginning of the pars pylorica. The smaller balloon lay at the lowermost end of the esophagus, often projecting well into the cardiac orifice.

In the preparation of turtles, viz., Pseudemys concinna, Pseudemys elegans, Graptemys geographica, and Chelydra serpentina, a method described by Carlson and Luckhardt (15) was followed. The stomach balloon was introduced through an incision in the esophagus in the neck. For recording contraction of the lung, usually the left, the corresponding bronchus was ligated, and a glass cannula communicating with the manometer was tied into the posterior tip of the organ. The vagus to be stimulated was isolated as a rule from the cervical sympathetic, ligated and cut.

In tracings taken from cats, the records from below upwards are the following: time and signals; esophagus-cardia; stomach and blood pressure. In all these tracings, time is recorded in 5 second intervals. In experiments on turtles, the stomach record is written above that of the lung. The vertical distance between the short horizontal lines in the figures measures the indicated volume-change in the corresponding balloon or organ. All tracings read from left to right.

RESULTS OBTAINED FROM CATS. The effects of excitation of the peripheral end of the vagus on the lower end of the esophagus and cardia of the cat depend on the frequency and intensity of stimulation. Single induction shocks, if relatively strong, or single make-break couples, produced by the tapping of a simple key, cause single contractions of a duration of about 10 seconds (fig. 7). As the frequency of stimulation increases, the intensity remaining constant, the excitatory effects become more pronounced. The single contractions fuse into a more or less evenly sustained contraction, which is often stronger than the single contractions alone, or there results an increase in tonus on which rhythmic contractions are superposed (figs. 6 and 7). The latter, as shown by direct observation, are peristaltic waves passing toward the stomach. With relatively strong stimulation, these pronounced excitatory effects occur with frequencies as low as 1 make-break couple for every 3 to 5 seconds. When the shocks are weaker, somewhat higher frequencies are required. The rhythm of the peristaltic waves, however, is quite independent of the frequency or intensity of stimulation.

When the frequency or intensity is increased somewhat beyond that required to give contraction during excitation of the nerve, the motor effects persist for some time after stimulation has ceased. The duration and magnitude of this after-response are proportional, within limits, to the duration of stimulation. They tend also to increase with increase in frequency and intensity of stimulation, the duration remaining constant, though this relationship was not studied carefully. The effect of prolonging the period of stimulation is shown in figure 5.

A decidedly different reaction occurs, however, when the frequency or intensity is increased beyond that required to give the motor effects

described. There is first an initial contraction, which is more brief the higher the frequency (fig. 6, *C* and *D*). This is followed by relaxation, the degree of which is more complete the higher the frequency or intensity (fig. 6, *B*, *D* and *C*). Little or no recovery or escape of the lower end of the gullet occurs, as a rule, from the effects of stimulation at this stage. The tonus level following the initial contraction may be indeed considerably lower than normal (figs. 6 *C* and 7). On cessation of stimulation pronounced after-contraction occurs, though this may be delayed in its appearance and reduced in extent with the higher frequencies (fig. 6, *C*). Peristaltic waves are often a marked feature of the motor after-effect. Repetition of stimulation while the after-contraction is taking place leads to relaxation, and this is followed on cessation of stimulation by after-contraction, the effect being similar to that obtained from the stomach (fig. 15). The relation of intensity of stimulation to the reaction produced is illustrated in figure 8.

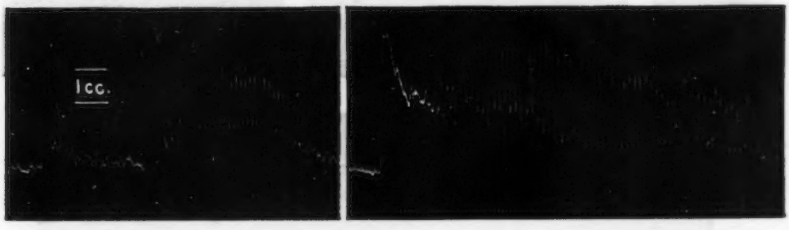


Fig. 5. Effect of duration of stimulation on extent of after-response of lower end of esophagus. Cat. Decerebrated by pithing. Vagi cut. Peripheral end of right vagus stimulated: coil A; 40 per second; 144 Z units. One-half original size. In this and other figures the vertical distance between the short horizontal lines measures the indicated volume change in the corresponding balloon or organ.

The frequencies required to produce the effects described above may vary considerably depending on the condition of the animal. In the experiment from which figure 6 was taken, the cat was anesthetized with chloralose, and a frequency of 40 per second, the intensity of the break shocks being 300 Z units, was hardly more than sufficient to cause relaxation after an initial contraction. The record shown in figure 7 was taken also from a cat anesthetized with chloralose, but about 2 hours previous to making the observations, it had been given 1 cc. of 1 per cent curare intravenously. When the experiment was carried out, the cat was breathing naturally, and the reflex hyperexcitability from the chloralose was evident. Under these conditions, the intensity of the break shocks being 540 Z units, the initial contraction and the following relaxation to a tonus level below normal were evoked by the low frequency of 1 per second. On cessation of stimulation, strong after-contraction occurred, the entire

reaction being quite typical of those produced usually by considerably higher frequencies.

During a single period of stimulation, the frequency remaining constant, it is possible to change contraction into relaxation by increasing the

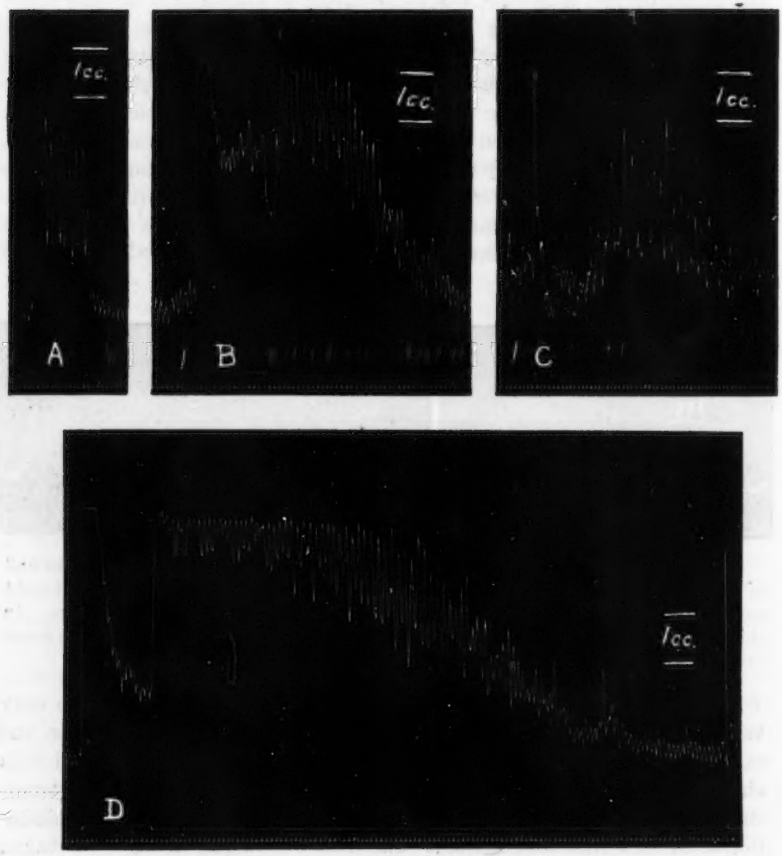


Fig. 6. Effect of stimulation of left vagus with increasing frequencies on lower end of esophagus. Cat. Chloralose. Vagi cut. Coil F; 300 Z units. A, 1½ per second; *B*, 20 per second; *C*, 240 per second; *D*, 40 per second. Maximum contraction indicates full collapse of balloon.

intensity. This change in reaction is shown in figure 8, which was taken from the same experiment as figure 7, but about an hour later. The frequency of stimulation during the taking of this tracing was 5 per second throughout. Considering the first observation, it is seen that a strength

of break shock of 350 Z units gave rise to pronounced and well-maintained contraction. On increasing the intensity to 430 Z units partial relaxation took place. Strong contraction occurred, however, when the strength of

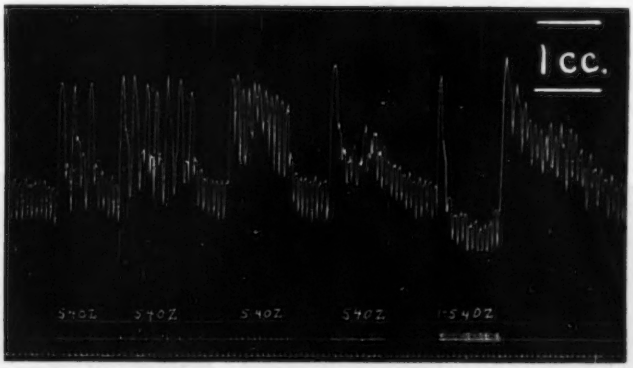


**Fig. 7. Effect of stimulation of left vagus with increasing frequencies on lower end of esophagus. Cat. Chloralose. Recovering from effects of curare. Coil B; 540 Z units.**

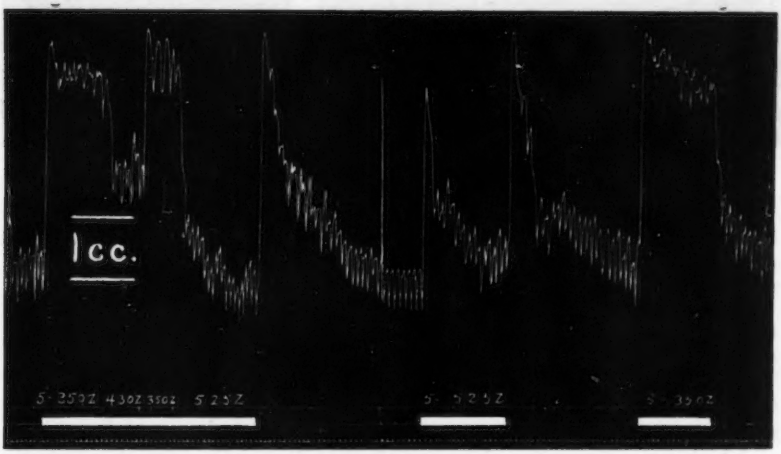


**Fig. 8. Effect of variation in intensity of stimulation of left vagus on lower end of esophagus. Cat. Chloralose. Recovering from effects of curare. Coil B; 5 per second; intensity as indicated.**

stimulation was diminished to its former value. Finally, increasing the intensity to 525 Z units led to relaxation to a tonus level considerably below normal. On cessation of stimulation, after-contraction occurred as usual.

The control observations on the right corroborate these results. The same effects are produced also by changing the frequency of stimulation, the intensity remaining constant. In figure 9, for example, it is shown that the lower end of the esophagus was thrown into well maintained contraction by a frequency of 2.5 per second. Increasing the frequency to 15 per second, however, led to relaxation to a tonus level below normal.

Deviation from these results is rare, though certain variations may occur. Occasionally the lower end of the gullet may recover to some extent from the inhibitory effects of strong stimulation. Such partial recovery

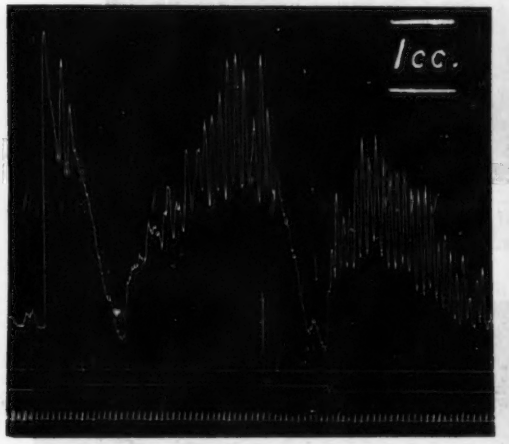


Fig. 9

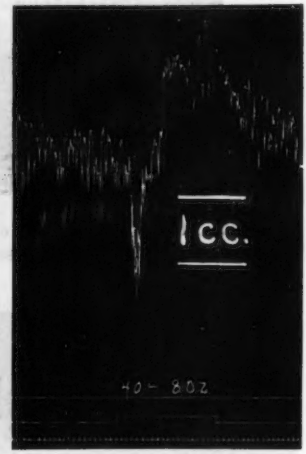


Fig. 10

Fig. 9. Effect of variation in frequency of stimulation of right vagus on lower end of esophagus. Cat. Chloralose. Vagi and left splanchnic major cut; left adrenal removed. Coil A; 370 Z units. Frequencies (changes indicated by upstroke in uppermost signal line): 2.5; 15; 2.5; 15 per second.

Fig. 10. Atypical effect of stimulation of left vagus on lower end of esophagus. Cat. Chloralose. Right vagus intact. Rotary interrupter. Coil B: 80 Z units; 40 per second.

during stimulation is indicated by a gradual rise of tonus, on which weak rhythmic contractions are superposed. A condition of peristaltic activity and high tonus, moreover, favors inhibition, but even under these conditions stimulation of sufficiently low frequency and intensity may have motor effects. It should be stated also that the initial contraction may fail to appear when one vagus is intact. In one experiment, furthermore, the right vagus being intact, the inhibition was limited to the beginning of the period of stimulation, pronounced contraction taking place thereafter. This atypical result is so similar to that which may be obtained from the

stomach on stimulation of the splanchnic major nerve in the cat that it is reproduced in figure 10.

Figure 11 shows that an intensity of stimulation, which as a rule causes inhibition of the active lower end of the gullet, may fail to do so if long continued, or if preceded in the same period of stimulation by a greater intensity. In fact, the weaker stimulation may appear to have an excitatory effect, though this may be due to post-excitatory action of the preceding higher intensity. That it is not due to local irresponsiveness of the nerve at the electrodes, however, is indicated by the fact that the weaker stimulation has its usual effect after a brief period of rest. In the one experiment in which paraldehyde was used as the anesthetic, moreover, strengths and frequencies of stimulation, which at first gave inhibition of the esophagus, as a result of frequently repeated periods of stimulation,

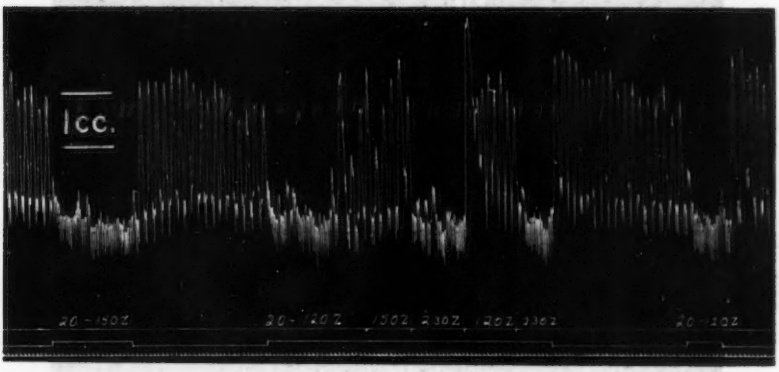


Fig. 11. Effect of prolonged stimulation, with variation in intensity, of left vagus on lower end of esophagus. Cat. Chloralose. Vagi cut. Rotary interrupter. Coil B: 20 per second; intensity as indicated.

soon caused only increased contraction. In this experiment there appeared to be a reversal of vagus action.

The difference in physiological effect between excitatory stimulation, of relatively low frequency and intensity, and inhibitory stimulation, of higher frequency and intensity, is shown clearly by simultaneous excitation of both vagi. Under these conditions, as shown in figure 12, inhibitory stimulation of one vagus may so reduce the contraction evoked by the opposite vagus as to make it almost negligible. On cessation of the inhibitory stimulation, the neuro-muscular mechanism gradually recovers its ability to respond normally. These results were obtained only from the lower end of the esophagus, and they were produced most readily in cats anesthetized with urethane or chloralose. It was observed at times, moreover, with urethane as the anesthetic, that simultaneous

excitatory stimulation of both vagi gave a total effect which was less in extent than the contraction caused by stimulation of either vagus alone.

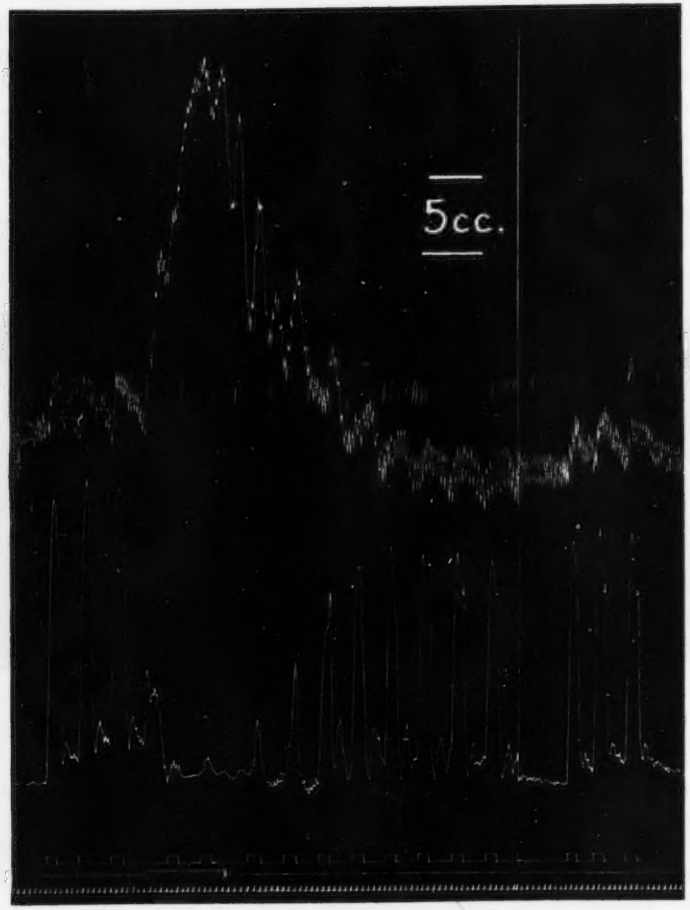


Fig. 12. Effect of inhibitory stimulation of right vagus (coil A: 370 Z units; middle signal) on excitatory stimulation of left vagus (coil G: 135 Z units; upper signal). Cat. Urethane. Records from lower end of esophagus and stomach; 40 breaks per second.

It was desirable to determine whether the different reactions of the esophagus and cardia to vagus stimulation were due to the presence in the vagus trunk of excitatory and inhibitory nerve fibers. To eliminate the possible participation of fibers of the thoracico-lumbar division of the

autonomic nervous system, the cervical sympathetic was removed with aseptic precautions from a level above the superior cervical sympathetic ganglion to a level below the inferior cervical ganglion. After sufficient time for degeneration, in one cat 16 days after the operation, stimulation of the corresponding vagus evoked the usual reactions. In other animals, the vagus was sectioned high in the neck, and no evidence of the presence of excitable fibers within it was obtained after time had been allowed for degeneration. In one case, stimulation 19 days after the operation was without effect. In another, the vagus having been cut above the ganglion nodosum 6 days previously to detect in addition the possible effects of antidromic impulses, stimulation was entirely ineffectual. There was no evidence, furthermore, of the persistence of inhibitory fibers longer than excitatory fibers. In one experiment, in which the ganglion nodosum had been removed 6 days previously, strong stimulation produced inhibition during stimulation, and this was followed by pronounced and long continued after-contraction. Nicotine in doses of 5 mgm. was found sufficient to abolish entirely the action of the vagus on the lower end of the esophagus.

The inhibitory effect of vagus stimulation, moreover, is not dependent on simultaneous inhibition of the heart. Figure 13 shows the effect of stimulation of the left vagus after its cardiac branches had been cut. The characteristic action of inhibitory stimulation is seen, though no appreciable change in heart rate, and only a slight fall in blood pressure occurred. Similarly the nullifying effect of inhibitory stimulation of one vagus on excitatory stimulation of the other is independent of inhibition of the heart. This was determined likewise by cutting the cardiac branches of the left vagus. Inhibitory stimulation of this nerve, thereafter, though having no noticeable effect either on heart rate or blood pressure, reduced greatly the the contraction caused by excitation of the right vagus.

Direct inspection of the lower end of the esophagus shows that the initial contraction preceding the dilatation caused by inhibitory stimulation may have the character of a peristaltic wave, but often it is a transitory contraction involving all of the lowest 2 cm. of the tube. The succeeding relaxation is not accompanied by a visible shortening of the longitudinal coat, which might be supposed to have a part in the increase in lumen. This muscular tunic, as well as the circular layer, appears to relax. The after-contraction involves the whole of the lower end of the gullet. It is similar to the reaction to excitatory stimulation of the vagus, consisting of a more or less pronounced tonic contraction, with peristaltic waves often superposed. No difference in reaction was observed between the cardia and the 2 or 3 cm. of the tube above it.

The reactions described above for the lower end of the esophagus and cardia of the cat correspond closely to those obtained in a similar manner

from the body of the stomach. When relatively low frequencies and intensities of stimulation are applied to the vagus, the stomach increases in tonus, and its peristaltic waves increase in strength (fig. 14). They

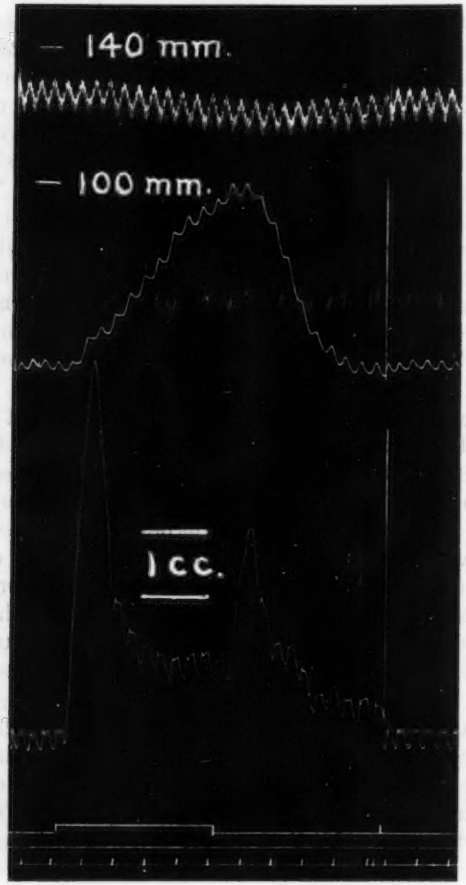


Fig. 13. Independence of effects of inhibitory stimulation of left vagus of inhibition of the heart. Cat. Urethane. Vagi cut. Coil G: 370 Z units; 40 per second. Records: lower end of esophagus; stomach; blood pressure.

take their origin also from a point nearer the cardia than normally. On cessation of stimulation, the motor effects gradually subside. The range of frequencies and intensities, which produce maintained contraction of the stomach, is considerably greater than that for the lower end of the

esophagus. Data were obtained, moreover, which indicate that the motor effects develop more quickly and persist longer, after cessation of stimulation, the greater the strength or frequency.

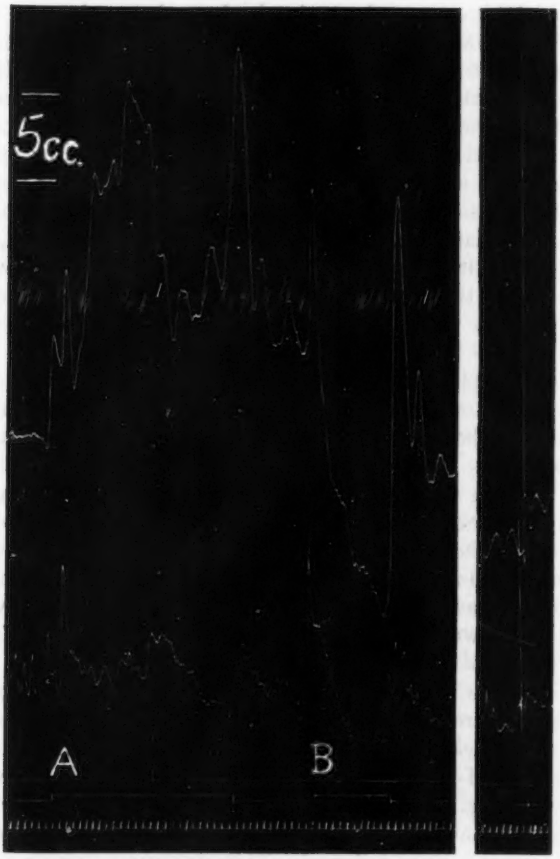


Fig. 14. Effects of excitatory and inhibitory frequencies of stimulation of right vagus on lower end of esophagus and stomach. Cat. Chloralose. Vagi, cervical sympathetics, and splanchnic majors cut; adrenals removed. Coil A; 415 Z units. Breaks per second: A, 2.5 to upstroke in uppermost signal line, then 12.5; B, 12.5.

Relatively higher intensities and frequencies are required, as a rule, to produce inhibition in the stomach than in the lower end of the gullet, provided that both vagi have been cut. The reaction, however, is much the same. An initial contraction occurs, and this is followed by relaxation,

often to a tonus level much below normal (figs. 14 and 15). There is a decided tendency, however, for the stomach to escape from the inhibition. It was found to begin to contract almost invariably when the excitation of the nerve was continued longer than 30 seconds, though as figure 14 shows, the inhibition may be maintained nearly a minute. This contraction begins slowly, but quickly increases to relatively great strength, and it persists for a considerable period after cessation of stimulation. If the excitation of the vagus is stopped during the period of relaxation, powerful after-contraction occurs, often persisting for a period of minutes (fig. 15). Repetition of the inhibitory stimulation during the after-response results in more or less complete relaxation, but the tendency to escape during the second period of stimulation is greater than in the first (fig. 15). The dilatation, produced by inhibitory stimulation, involves practically the entire stomach, being accompanied often by cessation of peristalsis. Both the motor and the inhibitory effects, evoked in the manner described, were confirmed by direct observation in the same animal.

Sectioning the splanchnic major nerves or pithing the spinal cord does not change the characteristics of vagus action either on the stomach or on the lower end of the esophagus. As in the case of the latter, moreover, so for the stomach, the vagus effects are independent of inhibition of the heart. In case one vagus is intact, however, and the stomach as a result is in a state of peristaltic activity and high tonus, stimulation of the peripheral end of the other vagus may produce relaxation with unusually low frequencies and intensities. This was the case in the experiment from which figure 16 was taken, and the reaction of the stomach was quite similar to that shown there for stimulation of the central end. The effect on the lower end of the esophagus, however, was motor.

Stimulation of the central end of the vagus, the other vagus being intact, has somewhat different effects on the lower end of the esophagus and the body of the stomach. The former relaxes as a rule during stimulation, but on cessation of stimulation, a single contraction or a series of contractions occurs (fig. 16). Only one observation was made in which contraction unquestionably occurred during stimulation, and even then a more marked motor after-effect followed. This observation was made in the same experiment from which figure 16 was taken, the motor effect being produced by a frequency of 20 per second and an intensity of 40 Z units. In the case of the stomach, however, pronounced dilatation occurs, the tonus and peristaltic activity being recovered gradually on cessation of stimulation (fig. 16). These effects are not appreciably affected by pithing the spinal cord. When both vagi were cut, however, and the cord and splanchnics were intact, the only unquestionable effect of central vagus stimulation was a slight dilatation of the stomach.

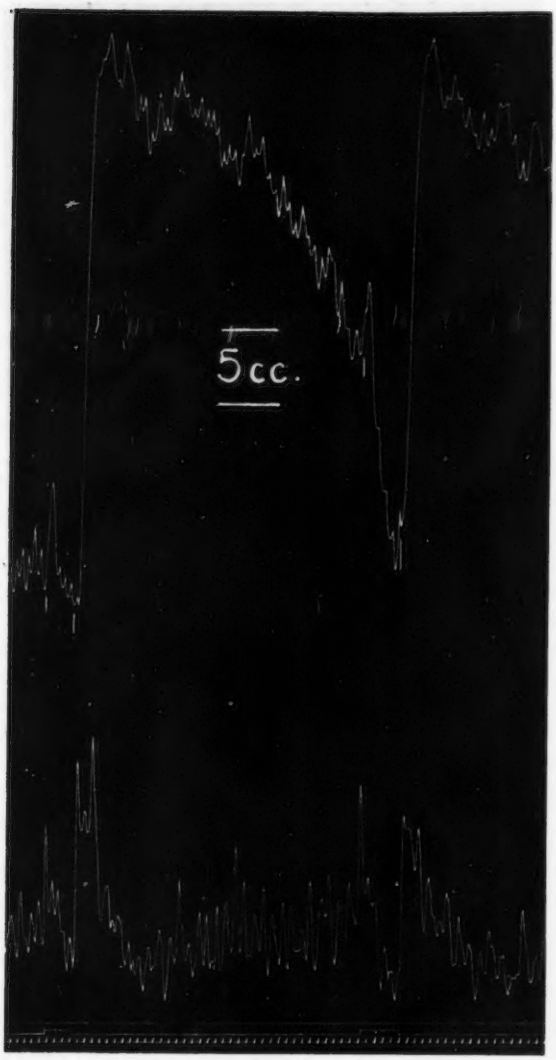


**Fig. 15.** Inhibitory effect of stimulation of left vagus with relatively high frequency and intensity on lower end of esophagus and stomach, showing especially inhibition of the after-contraction of the stomach. The vertical lines beneath the stomach record indicate duration of stimulation. Cat. Decerebrated by pithing; vagi cut. Coil A: 495 Z units; 40 per second.

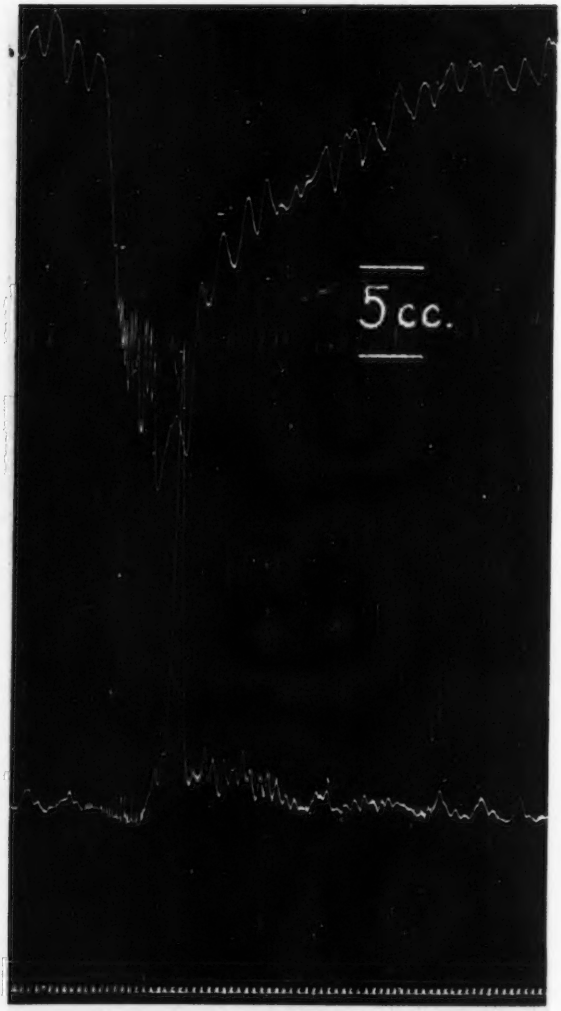


Fig. 16. Effect of stimulation of central end of left vagus on lower end of esophagus and stomach. Cat; decerebrated by pithing; cord pithed; right vagus intact. Coil A: 265 Z units; 15 per second.

It may be added that antiperistaltic waves were observed in two experiments in animals under chloralose. In one of them, both vagi had been ligated in the neck, and the lower end of the esophagus was exposed to view.

An occasional peristaltic wave was seen to pass orad. One such contraction, when it had nearly reached the oral end of the balloon, reversed its course and passed downward. In the other experiment, the reactions of the stomach to vagus stimulation were being watched after both vagi and both splanchnic majors had been cut, and it was observed that some of the peristaltic waves over the pars pylorica were directed orad. They appeared to stop in their course, however, on reaching the body of the stomach, in which the balloon was placed. Such reverse peristalsis, having been observed in only two experiments, must be regarded as unusual. It was observed at times also that the stomach was drawn upwards toward the diaphragm somewhat at the beginning of a period of stimulation. This was taken as evidence of contraction of the longitudinal muscular coat.

A few observations were made in addition on the action of atropin. Intravenous injection of 5 mgm. of the sulphate leads to cessation of peristaltic activity and profound loss of tonus both in the stomach and the lower end of the esophagus. There was some evidence, however, that the former recovered from the effects of the drug sooner than the latter. In one experiment, several minutes after injection, oscillations in the stomach tracing indicated slight rhythmic activity. In another, beginning recovery of the stomach was shown by slight contraction on stimulation of the vagus. In this experiment, however, the stomach remained inactive and atonic for more than an hour of observation. In both cases the lower end of the gullet, judging from its tracing, showed no signs of recovery.

RESULTS OBTAINED FROM TURTLES. The results obtained from the stomach of the turtle corroborated those already described for the cat. Stimulation of the peripheral end of the vagus with sufficiently low frequencies has a marked motor effect, exhibited as an increase in tonus, often accompanied by an increase in the magnitude of the rhythmic contractions in progress, as shown in figure 17. Relatively high frequencies, on the contrary, cause first a strong initial contraction; and this is followed by relaxation to a tonus level below normal and cessation of rhythmic activity, despite continued excitation of the nerve. On interruption of the stimulation, normal tonus and rhythmic activity are regained (fig. 18). It is possible to follow in the same turtle, by increasing the frequency of stimulation, the change from maintained motor effects to the initial contraction and subsequent relaxation. In an experiment on May 30, 1922, frequencies of 1 make-break couple in 15 seconds and 1 in 10 seconds caused an increase in tonus which persisted throughout the period of stimulation. In one observation, this period lasted 25 minutes. A frequency of 1 in 5 seconds, however, led to an initial contraction, which was followed by a gradual and progressive relaxation to a tonus level much below normal. The duration of the contraction was 6 minutes. On cessation of stimula-

tion, tonus was regained gradually. A frequency of 1 per second had a similar effect, but the initial contraction was stronger and it had a duration of only 3 minutes. The dilatation which followed it was correspondingly more pronounced. For 75 per second, the initial contraction was even stronger and more brief. Its total duration was not more than 1.5 minutes.

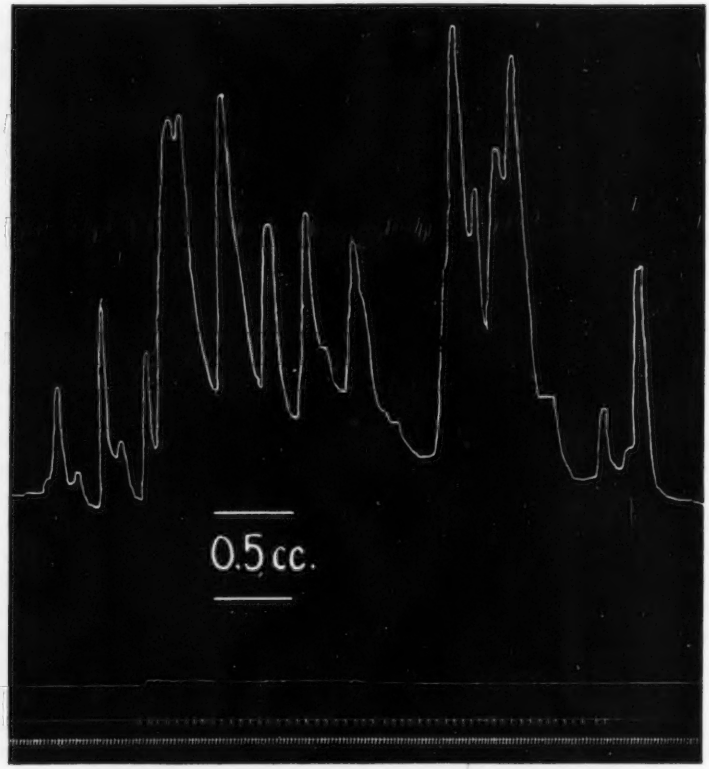


Fig. 17. Excitatory effect of low frequency stimulation of right vagus on stomach and lung of turtle. Coil B: 2,000 Z units. Time in 5 second intervals.

As a rule, the lung of the turtles used in the course of the work, with the exception of that of Chelydra serpentina, exhibits little or no tonus after section of the homolateral vagus nerve. Under these conditions, it responds with an evenly maintained contraction for relatively high frequencies of stimulation (fig. 18). In one experiment, however, the turtle having been decerebrated 18 hours previous to excitation of the nerve, the contraction decreased progressively for repeated periods of stimulation as

the frequency increased beyond 50 per second. At 150 per second, it could hardly be detected. There was no evidence of an initial contraction effect, the weaker contractions being well maintained throughout the period of stimulation. Coil B was used in this experiment, and the intensity of the break shocks was 140 Z units. With a freshly decerebrated turtle, the conditions of stimulation being practically the same (coil B, 145 Z units), frequencies of 360 per second and below gave maximal contractions.


Fig. 18. Effect of relatively high frequency stimulation of left vagus on stomach and lung of turtle. Rotary interrupter. Coil B: 110 Z units; 55 per second. Initial contraction of stomach not recorded in full. Time in 30 second intervals.

Figure 19 represents an unusual reaction. It was taken from an experiment in which the turtle, Chelydra serpentina, had been decerebrated 18 hours before stimulation of the vagus. The intensity of stimulation for the different observations was kept practically constant. Both vagi were isolated from the cervical sympathetics and cut. When the tonus of the musculature was low, a frequency of 1 make-break couple in 30 seconds had a decidedly motor effect, consisting of incomplete fusion of the single contractions set up by the stimuli. During a gradual rise of tonus, moreover, a frequency of 1 in 5 seconds halted the increase, keeping the tonus at a constant level throughout the period of stimulation. On cessation

of stimulation, the rise in tonus was resumed as if no interruption had occurred. Finally, when the tonus was high, as shown in figure 19, a frequency of 3 per second caused first a slight initial contraction, and this was followed by pronounced relaxation. It is of interest to note also that the tonus began to rise after 5 minutes of stimulation, despite continued excitation of the nerve.

The injection of 5 mgm. of nicotine, in one experiment, abolished vagus action on both the stomach and the lung.

Discussion. Reviewing the results described above, it is seen that the inhibition produced by the vagus is closely analogous to that studied by Wedensky in the nerve-muscle preparation of the frog. Low frequencies or intensities of stimulation are excitatory, whereas higher frequencies or intensities are inhibitory. The stronger the stimulation, the lower is the frequency required to produce inhibition, and similarly the higher the frequency, the lower is the intensity required for this effect. The inhibition, moreover, is preceded usually by an initial contraction, the duration of which bears an inverse relation to the frequency of stimulation applied to the nerve. These relations of character of stimulation to response produced hold also for the nerve-muscle preparation (29), (30), (23), (31). On the basis of similarity of reaction, therefore, it is probable that inhibition by the vagus is of the same fundamental nature as Wedensky inhibition. Confirmatory evidence for this view was obtained during the course of the work by Querido (32).

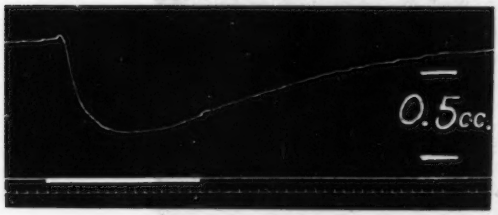


Fig. 19. Inhibitory effect of stimulation of left vagus on lung of turtle (see text). Coil B: 1,300 Z units; 3 per second. Time in 30 second intervals.

The observation of Carlson, Boyd and Pearcy (8) that inhibitory effects of vagus stimulation prevail when the tonus of the cardia is high, and that motor effects predominate when the tonus is low may find thus an explanation. Inasmuch as stimulation of the vagus may increase greatly the tonus of the cardia, it is probable that this condition is accompanied by the passage of propagated disturbances over a peripheral part of the neuro-muscular mechanism. Stimulation of the vagus when the tonus is high, therefore, would increase the frequency of propagated disturbances already in progress to an inhibitory value. When the tonus is low, on the contrary, nearly all of the disturbances would be produced by vagus action, and the frequency of these alone would be excitatory. It is

possible also that the observation of Bercovitz and Rogers (16), (17), that relatively low frequencies of stimulation cause inhibition of the stomach of the turtle, may have a similar explanation. The tendency for inhibition to occur more readily and for the initial contraction preceding it to fail to appear, when one vagus is intact, may be due likewise to an increase in an already considerable frequency of propagated disturbances in progress over a peripheral structure as a result of the action of the intact vagus. Such considerations may help to explain the regular occurrence of inhibition of the lower end of the esophagus and the stomach on stimulation of the central end of the vagus. The confusing statements of Openchowski (1), (3), however, are difficult to interpret, especially in the absence of tracings and a careful description of the preparation of the experimental animals.

The inhibitory reactions to vagus stimulation, moreover, are probably not due to the excitation of special inhibitory fibers in the nerve trunk. Experiments on nerve anastomosis (33), (34), (35) indicate that the action of nerve fibers is not dependent on the character of the impulses which they convey, but rather on their mode of termination. In the case of the vasomotor nerves, it has been assumed that the supposed dilator fibers are more responsive to weak or low frequency stimulation than the constrictor fibers (36). The reverse relation of effect produced to character of stimulation, however, holds for the vagus. The experiments on nerve degeneration, moreover, failed to reveal an admixture of functionally different fibers in the vagus, the results agreeing with those obtained by Dale, Laidlaw and Symons (37). It might be supposed, indeed, that Wedensky inhibition itself is due to stimulation of inhibitory nerves in the sciatic, but the experiments of Amaya (38) show that this is not the case.

In seeking to locate the particular part of the neuro-muscular mechanism which is responsible for the inhibitory reaction, a number of possibilities present themselves. Wedensky (39) and Hofmann (40) emphasized the importance of a refractory condition of the end-plate in Wedensky inhibition, and it might be expected, therefore, that synaptic regions would be involved. The work of Lucas (41) and Adrian (42) indicates that the relative refractory phase of the nerve fiber also is an important factor in its production. It is of interest to note in this connection also that Wedensky, in one of his most extensive papers (30), concluded that the seat of inhibition was the muscle fiber itself, though later he abandoned this view.[2]

The low frequency of stimulation with which the inhibition may be brought about under certain conditions, viz., 1 in 5 seconds for the turtle's stomach and 1 per second for the lower end of the esophagus of the cat

[2] Parts of Wedensky's Russian work were translated for the author by Dr. Leonard Kowarski of Warsaw, Poland.

(fig. 7), suggests at once that a relative refractory phase of the preganglionic fibers is not involved in the reaction, even though repetitive discharge as a result of strong stimulation (43), (44), (45) might take place. The nullifying effect of inhibitory stimulation of one vagus on excitatory stimulation of the other, moreover, shows definitely that the seat of inhibition lies beyond the preganglionic fibers. This is indicated also by the fact that the turtle's lung remains in a contracted state in response to vagus stimulation long after the stomach has relaxed. The synapse between the preganglionic fiber and the peripheral neurone is excluded also, for a block established there would not be expected to interfere with the functioning of more peripherally situated structures. The inhibitory effects of vagus stimulation after bilateral vagotomy, however, are manifested by loss of tonus and cessation of rhythmic activity of the smooth muscle. The structure which is responsible for the inhibitory action of the vagus, therefore, must be looked for beyond the preganglionic fiber and the synapse between it and the peripheral neurone.

If it is considered on the basis of the work of Magnus (46) that the spontaneous activity of the alimentary canal is dependent on the intact state of the nerve cells of the myenteric plexus, it might be expected that the inhibitory action of the vagus would be exercised in such a way as to reduce their activity, or to prevent their action on structures situated more peripherally. The experiments of Rogers and Bercovitz (17), indeed, led them to conclude that a refractory condition in the gastric nerve plexuses of the turtle could be produced by stimulation of the vagus. Evidence obtained by Gunn and Underhill (47), on the contrary, indicates that the spontaneous contractions of the digestive tract are myogenic. It would appear from their results that the seat of inhibition is to be found in the smooth muscle cells themselves. Results obtained recently by Dr. Jayme R. Pereira and the author, in stimulating skeletal, cardiac and non-striated muscle directly, favor the latter view. Relatively low frequencies of stimulation excite the different types of muscle to activity, whereas higher frequencies are inhibitory. In the case of the apex of the turtle's ventricle, for example, increasing the frequency to 10 breaks per second may suffice to stop the contractions set up by a lower frequency. After the application of atropin, however, increasing the frequency to 720 per second may fail to inhibit the rhythm. Further evidence of the same nature has been obtained by the author on stimulation of fibers coursing from the celiac ganglion to the stomach of the cat. The results of these experiments, however, must be left for fuller discussion in later papers.

Escape from inhibition, therefore, is to be accounted for on the basis of a decrease in the frequency of propagated disturbances set up within the muscle cell rather than within the peripheral neurone. The frequency

might be decreased to an excitatory value by the establishment of a condition of decrement in the region of a synapse, resulting in a diminution in the magnitude of the propagated disturbances passing through it. By repeated passage of such disturbances, a region of decrement would be expected to develop, inasmuch as a similar change takes place at the neuromuscular junction of the nerve-muscle preparation (48). A sufficient decrement, moreover, would render each propagated disturbance inadequate of itself to excite the more peripherally lying structure. Summation of these inadequate disturbances, on the analogy of summation of inadequate stimuli, might result in excitation of the peripheral structure, however, but at a lower frequency than that of the inadequate disturbances delivered to it. Thus the frequency of propagated disturbances in the peripheral structure might fall to an excitatory value. The condition of decrement leading to this result could be set up conceivably either at the synapse between preganglionic fiber and peripheral neurone, or between the latter and the muscle cell. Probably the development of such a state at synaptic regions is a causal factor in the escape of the heart from inhibition.

The occurrence of the motor after-response is to be interpreted as a manifestation of after-discharge of nerve impulses from peripheral neurones. This was shown to be true for the bladder by Langley (49), who obtained reactions on stimulation of the sacral nerves, after injections of nicotine and curare, which were quite similar to those described above for inhibitory stimulation of the vagus. No after-contraction was obtained, however, when purely post-ganglionic fibers were stimulated. Langley suspected that the unusual reaction to stimulation of the sacral nerves might be related to Wedensky inhibition. His results from frequency experiments, however, were negative, probably because the frequencies employed were too high for the degree of poisoning of the experimental animals (cf. fig. 7 supra). In speaking of the causation of the after-contraction, Langley states that it seems probable "that the after-contraction is due to an outburst of new impulses from the nerve cells and not to a freedom of action of impulses already in progress" (p. 179). In drawing this conclusion, however, he fails to consider the possibilities that the frequency of impulses over the post-ganglionic fibers may be much higher than that of the stimulation applied to the pre-ganglionic fibers, and that the after-contraction might have been due to a gradual decrease in the frequency of impulses over the post-ganglionic fibers through values excitatory for the smooth muscle. Doctor Pereira and the author found it necessary to apply some hundreds of stimuli per second to smooth muscle directly to obtain an inhibitory reaction.

The question arises, therefore, whether the after-discharge of nerve impulses is due to the continued action of single nerve cells or to a delay

of impulses in reaching their destination by long and circuitous nerve paths (50). It might be expected that such delay paths would extend from one collection of ganglion cells to another by association neurones, or that they might connect the nerve cells in a single ganglion with each other. There is evidence, however, against the existence of association neurones either between or within sympathetic ganglia (51), (52), (53). In the case of the stomach of the cat, moreover, in which the after-contraction may persist for a period of minutes, experimental evidence indicates that there is no extensive spreading of disturbances, set up by the vagus, from one part of the gastric nerve plexuses to another. It is well known that stimulation of the peripheral end of one vagus in the neck will cause contraction of the entire stomach, but Ducceshi (54) found that cutting one of the main branches of the vagus in the region of the cardia freed the corresponding part of the gastric musculature from vagus action.

The reaction of the lower end of the esophagus, the cardia and the body of the stomach of the cat to inhibitory stimulation of the vagus, therefore, may be accounted for as follows. Assuming on the basis of the work of Adrian (55) that conduction of propagated disturbances in the vagus and the peripheral neurones on its course is of an all-or-none nature, then increasing either the strength (42), (43), (44), (45) or the frequency of stimulation of the vagus, would have the effect of increasing the frequency of impulses over the nervous mechanism. In the peripheral neurones, the frequency of discharge might rise gradually rather than suddenly to a value depending on the frequency of impulses delivered to them from the pre-ganglionic fibers. As stated above, however, the frequency of disturbances over the post-ganglionic fibers is probably considerably greater than that over the pre-ganglionic. Likewise the frequency of propagated disturbances within the smooth muscle fibers would increase, passing first through an excitatory value which would cause the initial contraction. With further increase in their frequency, however, it is assumed that each travels in the relative refractory phase following its predecessor, being reduced thus in a conducting mechanism of the muscle fiber to a subthreshold value for the contracting mechanism. In the case of the nerve, such a reduction of the propagated disturbance as a result of travelling in the relative refractory phase produced by its predecessor has been shown quite definitely to occur (56), (48, p. 93). On cessation of stimulation, the frequency of disturbances over the peripheral neurones, and consequently over a conducting mechanism of the muscle fibers, would diminish, passing again through an excitatory value and giving rise to after-contraction.

Langley, in his paper on the bladder (49), discusses a number of reactions analogous to that obtained on stimulation of the sacral nerves, and suggests that they may be of the same fundamental nature in their causation. He

includes the reactions of the cardiac and anal sphincters of the rabbit to stimulation of the vagus and sacral nerves respectively, the response of the submaxillary gland of the dog and cat to stimulation of the chorda tympani and that of the dilator muscle of the pupil to excitation of the cervical sympathetic under certain conditions, the reversal of action of the vagus on the heart, and reflex rebound. It may be added also that the after-action of excitatory stimulation of the vagus, described above for the lower end of the esophagus, resembles closely after-discharge in spinal reflexes (57), (50). The relation of rebound to Wedensky inhibition has been considered recently by Forbes (50). The other reactions discussed by Langley, with the exception of reversal of action of the vagus on the heart, are so similar to that which is obtained from the alimentary canal on inhibitory stimulation of the vagus that the same considerations, in all probability, apply to them.

The reversal of action of the vagus on the heart, as described by Dale, Laidlaw and Symons (37), is obtained after the administration of a number of drugs, the effects of nicotine being discussed as typical. The drug itself reduces the rate of beat somewhat below normal. Stimulation of the vagus thereafter causes an initial slowing, but this is followed by a return during stimulation to the rate obtaining subsequent to the administration of the drug or to a moderate acceleration above it. On cessation of stimulation, an abnormal decrease in rate occurs, indicating delayed inhibition, but this passes off in the course of a few minutes. Stimulation during the period of delayed inhibition, however, may cause acceleration with a scarcely noticeable latent period, slowing again taking place on interruption of the stimulation. Dale, Laidlaw and Symons are inclined to favor the view that the acceleration is due to the action of accelerator fibers in the vagus, but, as Langley (49) suggests, the effect might be due to removal of slight excitation in the peripheral neurones. Evidence was obtained by Dale, Laidlaw and Symons that the delayed inhibition was due to vagus action, for atropin caused prompt acceleration to a rate equal to or above that preceding stimulation.

In view of the results described above for the alimentary canal, reversal of vagus action on the heart might be accounted for on the basis of *1*, a gradual increase in the frequency of propagated disturbances over the peripheral neurones on the course of the vagus; *2*, a gradual increase in the decrement suffered by these disturbances at the synaptic termination of the nerve fibers with more peripheral structures, or *3*, a combination of both factors. A gradual increase in the frequency of disturbances over the post-ganglionic fibers might result soon in considerable diminution in their magnitude as a result of each travelling in the relative refractory phase following its predecessor (56), (48, p. 93), (41). At the synaptic termination of the post-ganglionic fibers, moreover, the reduced disturbances would

be further diminished by decrement, or perhaps extinguished, with the result that the more peripheral structures would be freed from their effects. Inhibition would occur, however, at the beginning and after cessation of stimulation, as a result of the rise and fall respectively in frequency of disturbances over the peripheral neurones. If the frequency of impulses over the post-ganglionic fibers is constant, however, during stimulation of the vagus, a gradual increase in decrement at the terminal synapse might extinguish the propagated disturbances or render them subthreshold for the more peripheral mechanism. Inhibition at the beginning and after cessation of stimulation would occur as before as a result of a gradual increase and decrease respectively in synaptic decrement. It is probable that both factors work together, however, for the gradual passing off of the delayed inhibition suggests a gradual building up of a high frequency in the peripheral neurones at the beginning of stimulation. The increase in frequency of propagated disturbances is probably concurrent with an increase in synaptic decrement.

Attention may be called also to the fact that the reaction described above for stimulation of the central end of the vagus is purposive in its nature. The relaxation of the lower end of the esophagus and stomach is a condition which would favor the passage of food from the gullet to the receptacle below. The after-contraction of the former, moreover, occurs at a time when the stomach is fully relaxed, and ready to receive material, therefore, which might be pushed into it. The entire reaction recalls the receptive relaxation of the stomach described by Cannon and Lieb (13).

## SUMMARY

1. Stimulation of the peripheral end of either vagus nerve with relatively low frequencies or intensities has motor effects on the lower end of the esophagus, the cardia, and the body of the stomach of the cat. Stimulation with considerably higher frequencies or intensities has inhibitory effects on these structures, the inhibition being preceded as a rule by an initial contraction (figs. 5 to 9, 14 and 15). Similar results are given by the stomach and lung of the turtle in response to vagus stimulation (figs. 17 to 19). The physiological effectiveness of inhibitory stimulation of one vagus is illustrated for the lower end of the esophagus of the cat by its ability to reduce the effects of excitatory stimulation of the other vagus (fig. 12).

2. The reactions in the cat are independent of inhibition of the heart (fig. 13), and they are unaffected qualitatively by pithing the cord or cutting the splanchnic nerves (fig. 14). Experimental evidence indicates, furthermore, that they are not produced by the action of functionally different fibers in the vagus trunk.

3. In view of the close analogy of the reactions to Wedensky inhibition, they are accounted for on the basis of a reduction of propagated disturbances to a subthreshold magnitude in a conducting mechanism of the smooth muscle fibers, as a result of each travelling in the relative refractory phase produced by its predecessor.

4. The increased tendency for inhibition to occur when the smooth muscle is in a state of high tonus and rhythmic activity, therefore, is explained by an increase from an excitatory to an inhibitory frequency of the propagated disturbances already in progress over a conducting mechanism of the muscle fibers.

5. It is suggested that escape from inhibition may be accounted for, in part at least, on the basis of increased synaptic decrement, possibly associated with a decrease in the magnitude of propagated disturbances in the peripheral neurones.

6. It is probable that after-discharge from peripheral neurones is due to a tendency of the nerve cells when once set into action to cease their activity gradually.

7. A number of reactions obtained on stimulation of autonomic and sensory spinal nerves resemble the response produced by inhibitory stimulation of the vagus. It is suggested, therefore, that much the same considerations apply to them.

8. The reaction of the lower end of the esophagus, the cardia, and the body of the stomach of the cat to central stimulation of the vagus appears to be purposive in its nature (fig. 16).

9. Incidental observations on antiperistaltic waves, possible longitudinal shortening of the stomach, and the action of atropin are mentioned.

10. A moist chamber method for studying the innervation and movements of the viscera of mammals is described.

11. A non-oxidizable mercury key for the primary circuit is described (fig. 2).

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# THE EFFECT OF LONG-CONTINUED STORAGE AT LOW TEMPERATURE ON THE VITAMIN-A CONTENT OF EGGS

D. BREESE JONES, JOSEPH C. MURPHY AND OTTO MOELLER[1]

*From the Protein Investigation Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington*

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Modern improvements in industrial methods as applied to food products, such as refrigeration, dehydration, canning and other ways of preservation, have made it possible to keep foods in storage for relatively long periods of time. A large proportion of our food products is subjected to such storage before these products reach the consumer. It is therefore important to know what effect, if any, such treatment may have on the nutritive properties of the foods in question.

The work described in this publication was undertaken to ascertain the effect of long-continued storage at low temperature on the vitamin-A content of hens' eggs.

The recorded data on the effect of storage on vitamins are rather meager, and in many cases essential details are not given. Findlay (1), working with dry peas which had been kept for 38 years, found that they contained an appreciable quantity of vitamin B. He states that "in comparison with the observations of Ghose, however, they appear to have lost a small amount of their vitamin B content." Ghose (2) reported that 1 gram of peas furnished sufficient vitamin B to promote the growth of rats at a normal rate. Findlay found that .3 grams of the stored seeds were necessary to supply enough vitamin B to promote normal growth. Therefore, he concluded that some of the vitamin had been lost. Such a comparison can hardly be accepted as a safe basis from which to draw final conclusions.

Jensen (3) found that rice as paddi (rice in the ear) stored for 100 years contained nearly as much anti-beriberi vitamin as new rice, and that there was no perceptible difference between the vitamin-A content of the stored rice and that of fresh rice.

Experimenting with cod liver oil that had been stored for 6 months to 1 year, Holmes (4) states that "cod liver oil rendered from cod livers which have been stored at a low temperature and out of contact with the air has a higher vitamin-A content than that rendered from fresh livers."

[1] Some of the preliminary work in connection with this investigation was done by Mr. A. J. Finks, formerly of this laboratory.

Stiles (5) has reported that the vitamin A in butter is not destroyed by cold storage.

Storage of butter below 10°C. does not lower its vitamin A content, according to Drummond, Coward and Watson (6).

Results recently published by Wright (7) show that beef, mutton, lamb and pork after having been stored in a frozen condition for 2 to 9 years had suffered no change in vitamin B content. Later (8) the same author found that pork stored for 9 years at 2°F. to 15°F. was still active in vitamin A.

The results of our experiments with frozen eggs are in accord with those found by Wright working with frozen meats. After having been in storage in a frozen condition for nearly 9 years the eggs showed little, if any, diminution in vitamin A potency as compared with that of fresh eggs. The long period of time during which the eggs had been stored—a length of time to which food products designed for human consumption are rarely, if ever, subjected—constitutes a rigid test upon the effect of storage at low temperature on vitamin A.

The vitamin A content of the eggs was studied both by the prophylactic and by the curative methods.

MATERIAL. The storage eggs used in this investigation were first grade eggs laid in June and July, and were known as "July firsts." They were placed in commercial storage in the shell at 29° to 32°F., July 27, 1915. At the end of two months the eggs were broken out and their contents thoroughly mixed in a large lard can, and stored in a frozen condition at 0°F. or below, to 10°F.

After having been in storage in this condition for about 6½ years, the container was opened for some preliminary studies, and a bacteriological examination of the eggs made[2] by Miss Edmondson, who gave the following report:

| | |
|---|---|
| Total number of bacteria per cc. of egg | 1,440.000 |
| Number of viable bacteria per cc. of egg | 390,000 |
| No gas in lactose broth, in any dilution (dilution from x to x mm. inclusive) | |
| Growth of anaerobic bacteria up to m dilution. | |

Microscopic examination of undiluted egg and of aerobic and anaerobic cultures showed streptococci in short chains to be predominant. A few short, slender non-spore-forming bacilli were also seen.

The sample of eggs when taken had no objectionable odor and did not even after it was melted, and incubated at 37°C. overnight. Since the bacteriological examination showed no members of the colon group to be present, and the plate count was not great, the eggs seem to be in good condition.

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[2] We hereby express our thanks to Dr. C. Thom, Mycologist in Charge, Microbiological Laboratory, Bureau of Chemistry, and to Miss Ruth Edmondson, Assistant Mycologist, for their coöperation in making the bacteriological examination of the eggs here reported.

The final feeding experiments, the results of which are presented in this article, were begun in August, 1923, and although not completed until June, 1924, the eggs, which had then been in storage in a frozen condition for nearly 9 years, were still in good condition, and no change in their vitamin potency noted from that observed when the experiments were started.

Strictly fresh eggs were used for controls, which were obtained from a small nearby farm. They were laid in the summer, and were from hens that had free access to plenty of green food.

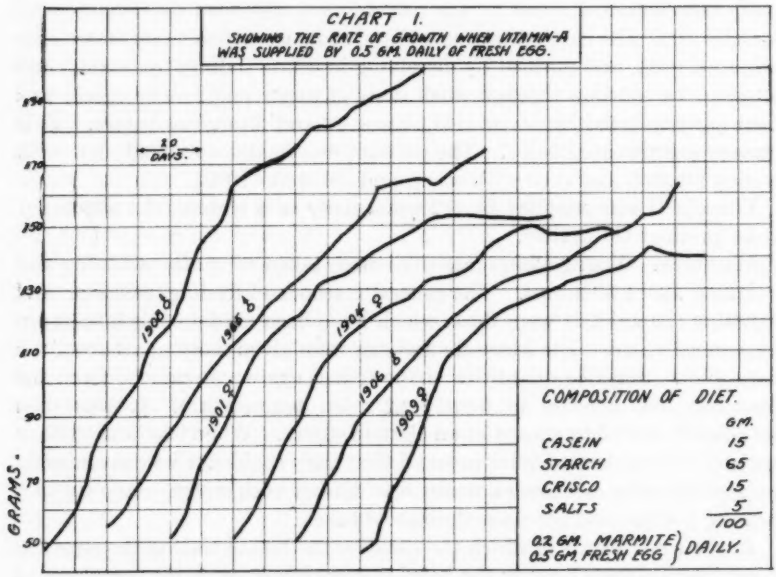
The composition of the basal diet is given on charts 1 and 2. The inorganic salts were furnished by the salt mixture described by Osborne and Mendel (9). The casein was precipitated at its isoelectric point from fresh skimmed milk, and purified by dissolving in dilute sodium hydroxide, and filtering the solution through thick mats of paper pulp. The casein was then reprecipitated by acetic acid, dissolved and filtered as before. This process was repeated twice. . The product was finally extracted, first with boiling alcohol, and then with ether, and dried at 110°C.

Vitamin B was supplied by 0.2 gram daily of a commercial autolyzed yeast product (Marmite).

METHODS. Young albino rats were used, taken soon after weaning and weighing about 50 grams. The general methods of feeding, weighing and handling the animals were those which have been used in this laboratory for several years. The Marmite and egg were given daily apart from the basal diet. Suitable quantities of the frozen egg were removed from the container and allowed to liquefy at room temperature. It was then thoroughly mixed by means of an electric stirrer. When the quantities of egg to be administered were so small that they could not be conveniently weighed directly, a known amount was diluted with water, made up to a definite volume and the desired aliquots taken.

In the experiments in which the curative method of testing the eggs was used, the rats were kept on the basal diet until unmistakable symptoms of xerophthalmia had developed. The efficiency of equal quantities of fresh and frozen eggs in curing the eye affection, as well as the effect on growth were compared. It is customary when testing for vitamin A by the curative method to keep the animals on the basal vitamin-free diet until a marked decrease, or cessation in the rate of growth, or even decline in weight occurs. The substance to be tested is then given and its vitamin potency estimated by its efficacy in causing a resumption of growth. It was believed that more conclusive results could be obtained by withholding the administration of the curative doses of the egg until xerophthalmia had developed, than by using merely the decline in weight as a criterion for starting the curative dosage. This method makes it possible to study the effect of vitamin A both as an anti-xerophthalmic and as a growth-promoting factor—functions which may be distinct and not strictly parallel.

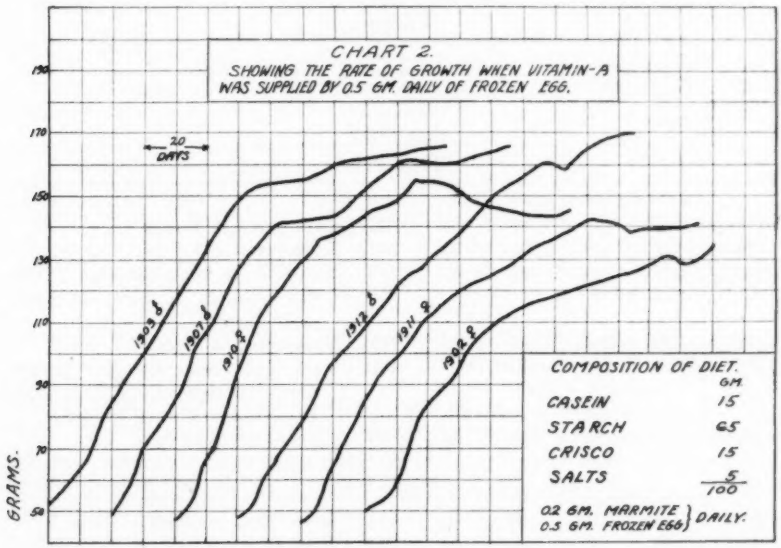
EXPERIMENTS BY THE PROPHYLACTIC METHOD. Preliminary feeding experiments showed that 0.75 gram daily of either fresh eggs or of the storage eggs given in addition to the basal diet enabled rats to grow at satisfactory rates for over 100 days. When the experiments were terminated the rats were still growing, and they showed no indications of nutritional deficiency. No difference in the rate of growth or in general appearance could be observed between the rats receiving the fresh eggs and those receiving the storage eggs.




In charts 1 and 2 are shown the rates of growth of rats receiving daily 0.5 gram of fresh and storage eggs. Rats 1908, 1905 and 1906, all males, receiving the fresh eggs made from fair to satisfactory growth for 120 days, and were still growing at as good a rate, or better, when taken off the diet. Rats 1901, 1904 and 1909, females, grew at a rate better than normal for 90 days, but at the end of that time their growth curves began to flatten. The rats receiving the storage eggs (chart 2), on the other hand, grew practically equally as well as did those receiving the fresh eggs for periods ranging from 60 to 120 days, but ultimately their rate of growth began to decline, and at the end of 120 days they were making little or no growth. Rat 1910, a female, had lost 10 grams during the last 50 days of the experiment.

Comparison of the results presented on charts 1 and 2 shows that 0.5 gram of fresh egg daily did not supply quite enough vitamin A, and that somewhat better results were obtained with the fresh eggs than with the storage eggs. Aside from the decreasing rate of growth of the rats receiving the storage eggs, toward the end of the experiments, no other indication of vitamin deficiency was noted in either lot of rats.

EXPERIMENTS BY THE CURATIVE METHOD.[3] In these experiments it was endeavored to determine the effectiveness of varying quantities of fresh and of storage eggs both in curing xerophthalmia, and also in causing resumption of growth in rats suffering from vitamin-A deficiency. The rats were kept on the basal diet until a decided xerophthalmia had




developed. The doses of egg were then given daily apart from the basal diet. It is of interest to note that with only one or two exceptions, all of the rats on the basal diet developed xerophthalmia in from 35 to 70 days.

The figures given in the tables refer only to the period during which the eggs were fed. Thus, the figures in the second columns represent the weight of the rats at the time the eggs were first given, and those in the third columns their weight at the end of the experiment.

[3] Some of the results obtained in the experiments with the fresh eggs which are here incorporated in the tables are included in an article entitled "The Vitamin A Content of Fresh Eggs," recently published by Murphy and Jones (10).

*Results with 0.5 gram daily of fresh and of storage eggs (table 1).* Comparison of the results obtained with 0.5 gram of fresh and storage eggs shows that this quantity given daily was sufficient to completely cure xerophthalmia in all cases, and to enable rats to resume growth at a satisfactory rate. The effectiveness of the storage eggs was evidently about equal to that of the fresh eggs at this level of intake.

In cases where xerophthalmia had not developed to a severe degree, the eyes were healed in a few days. Rat 1785, a female, had declined to such a degree that its eyesight was permanently destroyed, nevertheless it

TABLE 1

*Results showing the effects of feeding 0.5 gram daily of fresh and frozen eggs to rats having declined in weight and suffering from xerophthalmia as a result of vitamin-A deficiency*

| RAT NUMBER | WEIGHT WHEN EGGS FIRST GIVEN | FINAL WEIGHT | GAIN | DAYS GIVEN EGG | FOOD INTAKE DURING PERIOD OF EGG FEEDING | REMARKS |
|---|---|---|---|---|---|---|
| | | | Fresh eggs | | | |
| | *grams* | *grams* | *grams* | | *grams* | |
| 1781♂ | 76 | 98 | 22 | 35 | 222 | Eyes cured in 4 days |
| 1782♂ | 84 | 118 | 34 | 35 | 240 | Eyes cured in 7 days |
| 1783♂ | 92 | 118 | 26 | 35 | 249 | Bad case of xerophthalmia. One eye cured in 14 days, the other in 28 days |
| 1784♂ | 76 | 104 | 28 | 35 | 199 | Eyes cured in 4 days |
| 1785♀ | 68 | 100 | 32 | 35 | 191 | Very bad case. Eyesight permanently destroyed. Rat brought to otherwise normal condition |
| 1786♀ | 62 | 83 | 21 | 35 | 160 | Eyes cured in 14 days |
| | | | Storage eggs | | | |
| 2013♀ | 64 | 112 | 48 | 35 | 173 | Eyes cured in about 14 days |
| 2014♀ | 104 | 136 | 32 | 35 | 234 | Eyes cured in 10 days |
| 2015♂ | 106 | 137 | 31 | 35 | 252 | Eyes cured in about 7 days |
| 2047♂ | 68 | 94 | 26 | 35 | 173 | Eyes cured in 10 days |

responded promptly to the egg treatment; and at the end of 35 days had gained 32 grams and was in a state of fair nutrition.

*Results with 0.25 gram of fresh and of storage eggs (table 2).* When the quantity of egg was reduced to 0.25 gram daily, little, if any, difference in the vitamin-A potency between the fresh and the storage eggs was apparent, either in causing the resumption of growth or in the curing of xerophthalmia. Compared with the results obtained with 0.5 gram of the eggs, the rats made slightly poorer growth and the time required for curing xerophthalmia was decidedly longer. Although rat 1808 receiving fresh

eggs, and rats 1991 and 1993 receiving storage eggs resumed growth at a fair rate, they were not completely cured of xerophthalmia, however, even at the end of the experiment.

*Results with 0.1 gram of fresh and of storage eggs (table 3).* The results given in table 2 indicate that 0.25 gram of the eggs given daily is about the lower limit at which they are effective in curing xerophthalmia. Small differences in the vitamin content of given substances can be best detected when the materials to be tested are fed in quantities at, or slightly below

TABLE 2

*Results showing the effects of feeding 0.25 gram daily of fresh and frozen eggs to rats having declined in weight and suffering from xerophthalmia as a result of vitamin-A deficiency*

| RAT NUMBER | WEIGHT WHEN EGGS FIRST GIVEN | FINAL WEIGHT | GAIN | DAYS GIVEN EGG | FOOD INTAKE DURING PERIOD OF EGG FEEDING | REMARKS |
|---|---|---|---|---|---|---|
| | *grams* | *grams* | *grams* | | *grams* | |
| **Fresh eggs** | | | | | | |
| 1799♂ | 71 | 101 | 30 | 35 | 224 | Eyes cured in 4 days |
| 1809♂ | 62 | 81 | 19 | 35 | 207 | Eyes cured slowly. Slightly sore on 28th day. Cured by 31st day |
| 1811♂ | 70 | 100 | 30 | 35 | 211 | Both eyes cured in 10 days. Slight relapse on 25th day. Healed again |
| 1808♀ | 68 | 91 | 23 | 35 | 174 | Eyes slightly sore at the end of the experiment |
| 1810♀ | 69 | 81 | 22 | 35 | 167 | Eyes cured in 10 days |
| 1812♀ | 80 | 90 | 10 | 35 | 190 | Eyes cured within a week |
| **Storage eggs** | | | | | | |
| 1990♀ | 68 | 104 | 36 | 35 | 184 | Eyes cured in 7 days |
| 1991♀ | 66 | 94 | 28 | 35 | 225 | Bad case of xerophthalmia, one eye slightly sore at end of experiment |
| 1992♀ | 85 | 102 | 17 | 35 | 152 | Eyes cured in 17 days |
| 1993♀ | 96 | 118 | 22 | 35 | 201 | Not entirely cured at end of experiment |

the minimum needed to supply the vitamin requirements of the animals. Another series of experiments was therefore started in which 0.1 gram of the eggs was given. Although the growth recovery of the rats getting the 0.1 gram doses was nearly as good as that of the rats receiving 0.25 gram, the effectiveness of 0.1 gram for curing xerophthalmia was very slight. Only one rat was completely cured by the fresh egg even after three weeks of feeding. The others gave no indication that they would ever fully recover on this quantity. Growths at a slower rate resulted with the

storage eggs than with the fresh eggs. The effectiveness of the storage eggs for curing xerophthalmia was also somewhat less than that of the fresh eggs. Although rat 1888 died 21 days after having first received the storage eggs, it had gained 12 grams during that time and the condition of its eyes was improving at the time of its death. The onset of xerophthalmia in the case of rat 1885 was so sudden and severe that it was unable to respond when given the eggs.

TABLE 3

*Results showing the effects of feeding 0.1 gram daily of fresh and frozen eggs to rats having declined in weight and suffering from xerophthalmia as a result of vitamin-A deficiency*

| RAT NUMBER | WEIGHT WHEN EGGS FIRST GIVEN | FINAL WEIGHT | GAIN | DAYS GIVEN EGG | FOOD INTAKE DURING PERIOD OF EGG FEEDING | REMARKS |
|---|---|---|---|---|---|---|
| | | | Fresh eggs | | | |
| | *grams* | *grams* | *grams* | | *grams* | |
| 1855♂ | 62 | 85 | 23 | 35 | 206 | Not quite cured of xerophthalmia at end of experiment |
| 1856♂ | 79 | 107 | 28 | 35 | 226 | Eyes cured in 21 days |
| 1858♀ | 72 | 98 | 26 | 35 | 231 | Eyes in bad condition at end of experiment |
| 1859♀ | 97 | 121 | 24 | 35 | 244 | Eyes slightly sore, at end of experiment, but improving |
| | | | Storage eggs | | | |
| 1884♂ | 83 | 101 | 18 | 35 | 187 | Eyes completely cured in 10 days. Relapsed. Slightly sore at end of experiment |
| 1886♂ | 77 | 94 | 17 | 35 | 164 | Eyes cured in 14 days |
| 1888♂ | 74 | 79 | 5 | 21 | 103 | Died suddenly on 21st day. Eyes were improving, and had gained 12 grams in weight |
| 1885♀ | 87 | 69 | −18 | 4 | | Avitaminosis too far advanced for rat to respond to egg. Died on 4th day |
| 1887♀ | 96 | 91 | −5 | 35 | 155 | Eyes somewhat sore at end of experiment. Developed abscess on throat |

## CONCLUSIONS

The results of these feeding experiments show that no serious deterioration had taken place in the vitamin A content of the eggs which had been held in storage for 9 years in a frozen condition. In 0.25 gram daily doses, the storage eggs were found to be as effective as fresh eggs in curing

xerophthalmia. Even 0.1 gram daily caused noticeable improvement in the condition of the eyes, arrested the decline in weight, and caused a moderate resumption of growth which continued for 2 to 5 weeks. The somewhat poorer results obtained with the storage eggs than with the fresh eggs suggest that a slight deterioration may have taken place during the period of storage. However, when the difficulties and possible sources of error involved in the present available methods for estimating vitamin A are considered, such slight differences as were found in the vitamin A content of the fresh and storage eggs cannot be of much significance.

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# COMPARATIVE STUDIES ON PUPILLARY REACTION IN TETRAPODS

THEODORE KOPPÁNYI AND NELLES B. LAUGHTON

*From the Hull Physiological Laboratory of the University of Chicago*

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In the vertebrate phyla, the fishes show an immovable iris which cannot be affected by light or any drugs; only the pupil of the Tetrapods show definite pupillary reaction. It is possible in these animals to distinguish two types of pupillary reactions, namely, a local reaction and a central reaction. It is generally considered that the warm-blooded animals have a central reflex only and the amphibians merely a peripheral reflex.

One of the most important discoveries concerning the pupillary reaction was the work of Arnold (1) and of Brown-Sequard (2), who showed independently that light can affect the iris of the enucleated frog eye. This observation was confirmed by many workers and it was generally stated that the amphibians have only a local pupillary reflex and there was no direct evidence that in these forms there existed a central reflex. This conclusion was also reached in a recent paper by Murase (3).

The present work was undertaken primarily to determine if there is in the frog a central reflex mechanism controlling the pupil, besides the local mechanism.

Lewandowsky (4) discovered that dilatation of the pupil resulted from intravenous injection of adrenalin. His observation was confirmed by Langley (5) and Boruttau (6), the effect being very evanescent, lasting less than one minute. Meltzer (7) showed that the action of adrenalin was directly on the "receptive substance" of Langley. Lichtwitz (8) severed all structures in the thigh of the frog with the exception of the sciatic nerve, thus making a preparation in which the leg was connected to the trunk only by the sciatic nerve. He injected adrenalin intramuscularly into the leg in such preparations and in a large percentage of the cases obtained dilatation of the pupil within 10–85 minntes. He interprets this as evidence of passage of adrenalin through the nerve trunk.

1. We exposed the brain of the frog, including the medulla, and very weak faradic currents were applied to the forebrain, optic lobes, cerebellum, medulla and the optic tract. In all cases bipolar electrodes were used for stimulation. Stimulation of the forebrain, cerebellum and medulla produced no change in the size of the pupil. *Stimulation of the*

*optic lobes, however, always resulted in a marked dilatation of the pupil of both eyes.*

The eye was removed from the orbit, care being taken not to injure the optic nerve in any way. Faradic currents were applied to the optic nerve with bipolar platinum electrodes and the reaction of the pupils was observed. At no time during the stimulation of the nerve or following the stimulation was there any change in the size of the pupil. Hence the only positive effects observed on the pupillary reaction was obtained when the optic lobes were stimulated.

2. Preparations of the frog were made similar to those used by Lichtwitz as described above. The effects on the pupil of injection of adrenalin, pituitrin, chloretone, warm water and ice water intramuscularly into the isolated leg were studied.

Not infrequently the injection of adrenalin caused marked dilatation of the pupil within a few minutes of the injections. In some of the experiments, however, when large amounts of adrenalin were used there was a marked constriction of the pupil. The injections of pituitrin, chloretone, hot and cold water into the isolated leg were without any effect on the pupil. Stimulation of the sciatic nerve with faradic currents gave doubtful results.

3. It has long been known that injection of adrenalin and pituitrin into the conjunctival sac of mammals results in a marked dilatation of the pupil. The idea occurred to us that mechanical pressure might account, in part, for this reaction. We therefore injected adrenalin, pituitrin, hot and cold water, and, following the suggestion of Doctor Lim, air into the conjunctival sac of the rat. In every case there was a marked dilatation of the pupil. That the dilatation was not due to pain was indicated by the absence of the dilatation when the sac was punctured with a needle. After these injections in the albino rats the pupil remained in the dilated condition for approximately six hours, when it attained its normal size.

These experiments were repeated on frogs, but following the injection of water or air into the conjunctival sac, there was no change in the pupil.

Discussion. The consensual pupillary reaction in some mammals serves as one direct proof that there is a central reflex mechanism involved in controlling the pupillary reactions in those forms. This consensual pupillary reaction has never been demonstrated in amphibia. It has, therefore, been claimed that there is no central reflex mechanism involved in the control of the pupillary reaction in the frog.

The presence of the local mechanism in the amphibia does not preclude the presence of a central nervous mechanism having a direct action on the pupils. Our results show that stimulation of the optic lobes of the frog with weak faradic currents causes marked bilateral dilatation of the pupil. It therefore seems probable that the optic lobes and midbrain act

as part of a reflex mechanism for pupillary dilatation. Our observations thus indicate that in the frog there is a central mechanism for pupillary reactions as well as a local mechanism.

Our results, using the Lichtwitz preparation, confirm the observations of Lichtwitz. Further, our results show that the pupillary reaction is specific for adrenalin as injections of other drugs, or hot and cold water had no effect on the pupil. Furthermore, it is evident that the dilatation produced by the injection of adrenalin is not produced by the mechanical stimulation of the nerve fibers in the leg. It must either be due to the passage of adrenalin along the sciatic nerve or to a specific chemical stimulation of the nerves by adrenalin.

The dilatation of the pupil following injection of air or water into the conjunctival sac is probably a central reflex initiated by pressure stimulation of afferent nerves in the distended parts. Whether these nerves are pain nerves so that the reaction is only an instance of pupil dilatation from pain must be determined by another line of experiments.

## SUMMARY

1. Stimulation of the optic lobes in frogs with faradic currents causes dilatation of the pupils.

2. Adrenalin sometimes gives positive results in the Lichtwitz preparation. Other drugs had no effect on the pupil in similar preparations.

3. Injection of water or air into the conjunctival sac of albino rats causes dilatation of the pupil lasting for four to six hours. Injection of water and air into the conjunctival sac of frogs produces no change in the size of the pupils.

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# SHOCK FROM FAT EMBOLISM OF THE VASOMOTOR CENTRE

W. T. PORTER

*From the Laboratory of Comparative Physiology in the Harvard Medical School*

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## I. INTRODUCTION

A soldier in a first-line trench is struck by a fragment from an exploding shell. The femur is broken. Minutes pass, sometimes an hour or two, at most a very few hours, and even the stretcher bearers see that the wounded man is in a state of shock. He looks it. He is utterly relaxed; pale as the dead; his eyes are pallid and sunken; he is apparently but not really unconscious; his breathing is shallow and frequent; his heartbeat rapid and feeble; his pulse is scarcely to be felt at the wrist; and when the diastolic arterial pressure is taken, it is found to be dangerously low, 60 mm. or thereabouts. It is with this early traumatic shock that these pages chiefly deal—an affair of the *postes de secours* and the field hospitals well within the zone of fire.

These pages will demonstrate that among the causes of early shock is one not previously known—fat embolism of the vasomotor region.

It will be shown that injuries of the long bones are in war a very frequent occasion of shock, probably the most frequent occasion. It will be pointed out that numerous investigations by earlier observers prove that wounds of the long bones set free considerable quantities of fat and that this fat is found post mortem in the vessels of the lungs, brain and other organs.

Experiments first made by the present writer will be cited to prove beyond question that the symptom complex of shock may readily be produced by very small quantities of fat injected into the bulbar vasomotor region through the vertebral arteries. A photomicrograph will be exhibited in which fat thus injected is seen in the capillaries of the bulb. And, finally, shock will be prevented by guarding the vasomotor region against the entrance of fat, although the lungs and other organs are strongly embolized. Upon this evidence the profession will be asked to accept a new surgical entity; namely, fat embolism of the bulbar vasomotor region as a cause of the shock which so often promptly follows wounds of the long bones.

Fat embolism of the vasomotor centre being thus established as a cause of shock, we shall clear away certain misconceptions: we shall show why

emboli passing to the brain through the carotid arteries often fail to produce shock; we shall demonstrate that pulmonary embolism does not in itself cause shock; we shall prove that the hypothesis of exhaustion of the vasomotor centre is an error, based in part on the misuse of the absolute reflex change in blood pressure as contrasted with the percentile change. Were these misconceptions allowed to stand, fat embolism of the vasomotor centre might be denied or at least its true position confused. These errors refuted, the way is open for the recognition that shock is but a phase common to widely different pathological states. It will then be proved that these pathological states fall into two groups; one in which the vasomotor centre is normal, and one in which this centre is directly attacked. Each of these pathological states will be given its place relative to fat embolism of the vasomotor centre. In conclusion, the reader will be asked to consider a systematic treatment of shock, based on new principles, and found successful during the Great War.

## II. EARLY SHOCK IS MOST FREQUENT AFTER SHELL WOUNDS OF THE LONG BONES

Let us determine first what injuries are in war most often followed by shock. Surgeons of experience will agree that shell fracture of the femur is often followed by shock, so often as to suggest in many minds a causal relation. For example, the surgeons of the Carrel hospital in Compiègne, Military Hospital No. 21, to which I was attached in August, 1916, told me that shell fracture of the femur was the most frequent cause of shock. The opinion is widely held. Such opinions may be supported by recorded evidence, statistically sound; or they may rest on the stored memory of many cases personally observed. Both supports are worthy of respect. In 1916 sound statistical evidence was lacking; at that time, trustworthy records of the number of cases of early shock per thousand casualties probably did not exist. At any rate, I was unable to learn of such records. Statistics are seldom, if ever, the business of an advanced dressing station under fire. It was therefore my duty to get first-hand knowledge of the incidence of shock among the freshly wounded. The opportunity did not arise until the following year.

On May 22, 1917, I found myself about 300 metres from the crest of Mont Blond, one of the summits in the Massif de Moronvillers, a long ridge which commands the plain beyond Châlons-sur-Marne. The French had won all this important position except the part above my station. At six o'clock, May 25, the French took this remaining portion by storm. Many freshly wounded men passed through my poste. I watched the blood pressure carefully. The wounds were often grave, but there was no shell fracture of the femur and no shock.

The several *postes de secours* on the Massif de Moronvillers sent their wounded to a sorting station or *triage* at Mourmelon-le-petit, the nearest village in the plain of Châlons. In the last days of May, I saw at this *triage* more than one thousand freshly wounded men. Aside from a few abdominal cases, in which there was probably direct injury to the vasomotor nerves of the abdominal vessels, the only shock was that caused by fracture of the femur or by multiple wounds through the subcutaneous fat —conditions in which fat embolism is known to take place.

From June 23 to June 30, 1917, I was at Vauxtin, seat of a *triage* near the Chemin des Dames. Here again was the clearest evidence that early shock is rarely to be seen except after shell fracture of the long bones or where the vasomotor nerves of the abdomen are injured.[1]

These personal observations on more than a thousand consecutive casualties fresh from the front line trenches show that shell fracture of the long bones is the most frequent cause of early shock. They support the view of many distinguished surgeons.

Material from hospitals behind the lines is excluded from this discussion, because of the errors arising from the inevitable infection and from the various evils of transport and delay. Less cautious students may consult publications 25 and 26 of the Medical Research Committee, London, 1919. They will there find lists in which shell fracture of the long bones is again the most frequent cause of shock.

### III. INJURIES OF THE LONG BONES CAUSE FAT EMBOLISM

That wounds of the long bones set free considerable quantities of fat has long been known. Indeed, the number of such cases is so great[2] that we need cite here only a few illustrative instances.

Godlee and Williams[3] examined two fractures of the femur from the Willesden railway accident. In one, fat emboli were found in the brain, heart, lung and kidney. In the other, Sudan III showed the capillaries of the brain and lungs "blocked with fat."

LeCount and Gauss[4] found fat emboli in the lungs in each of 14 cases of fracture (4, femur; 3 humerus; 5, tibia and fibula; 1, calcaneous; 1, pelvis). In four of these cases a microscopical examination was made; in each of the four, fat emboli were seen in the brain.

Bissell[5] determined the fat in the blood in thirty-one persons discharged from Cook County Hospital, Illinois, as "cured" from various maladies

[1] Boston Med. and Surg. Journ., 1917, clxxvii, 326–328.

[2] The literature of shock now carries more than a thousand titles. Such a mass could not be treated adequately within the limits of this paper.

[3] Godlee and Williams, Lancet, 1911, i, 1062.

[4] Le Count and Gauss, Trans. Chicago Pathological Society, 1915, ix, 251–258.

[5] Bissell, Journ. Amer. Med. Assoc., 1916, lxvii, 1926–1927.

other than injuries or operations; the average fat content was 0.442 per cent. He then measured the fat content of the blood in ten cases of fracture; the average fat content was 2.5 per cent. The experimental error in the method used was found to be 0.1 per cent.

Mott and Uno[6] examined the brain in four cases of shock after fracture of the humerus, femur, tibia, and the tibia and fibula, respectively. Fat emboli were found in the brain in each case.

Fat embolism also follows surgical interference with the bone marrow; for example, the forced straightening of bones.

The same observation has been made in numerous experimental studies. It is, in short, generally agreed that fat embolism is produced by *a*, experimental fracture of the long bones; *b*, jarring of the bones without fracture; *c*, injuries to the bone marrow. These results have often been obtained without damage to the soft parts.[7]

It is also well known that wounds of the subcutaneous fat are often followed by fat embolism.

The critical reader will note that the evidence thus far establishes the following propositions. Fracture of the long bones is a common accident in peace and war. This injury is very frequently followed by shock, always dangerous and often mortal. Large quantities of fat may enter the blood stream after fracture to be found post mortem blocking the vessels of the brain and other organs. But clinical evidence does not prove that fat embolism is a cause of shock. It is true that the testimony just given makes fat embolism a probable cause, a probability strengthened by the shock which has followed certain operations upon tissues rich in fat. Against this probability stands the fact that in no clinical case is fat embolism the only factor at work. Proof demands that the several possible causes be separated, and that shock be produced by fat embolism alone. Experiments on animals are required. Such experiments will now be presented.

## IV. FAT EMBOLISM OF THE VASOMOTOR CENTRE PRODUCES SHOCK

It has been shown above that early traumatic shock most often follows injuries of the long bones; that such injuries discharge into the blood stream very considerable quantities of fat; and that the resulting emboli are found in the lungs, the brain, and other organs. It is necessary now to prove that this fat embolism is *per se* the cause of the shock observed after injuries of the long bones and thus the most frequent cause of early shock.

[6] Mott and Uno, Proc. Royal Soc. Medicine, 1922, xv, Neurology, 25–40.

[7] Two recent papers of interest are Caldwell and Huber, Surgery, Gynec. and Obstetrics, 1917, xxv, 650–663; and E. Ziemke, Deutsch. Zeitschr. f. d. gesammt. gericht. Med., 1922, i, 193–203.

TABLE 1

*First experiments on the injection of oil into the venous circulation*

| DATE | ANIMAL | DIASTOLIC CAROTID PRESSURE | | NATURE OF EMBOLI |
|---|---|---|---|---|
| | | Before injection | After injection | |
| *1917* | | *mm.* Hg | *mm.* Hg | |
| February 2 | Cat | 110 | 30 | Emulsion of cod liver oil and acacia in jugular vein |
| February 3 | Cat | 115 | 35 | Thick cream in jugular vein |
| February 5 (1) | Cat | 140 | 40 | Thick cream in jugular vein |
| February 5 (2) | Cat | 130 | 25 | Neutral cottonseed oil in jugular vein |
| February 6 | Cat | 105 | 28 | Neutral olive oil in jugular vein |
| February 7 | Cat | 140 | 70 | Neutral olive oil in jugular vein |
| February 8 | Cat | 130 | 63 | Neutral olive oil in jugular vein |
| February 12 | Cat | 80 | 32 | Neutral olive oil in jugular vein |
| February 13 | Dog | 140 | 40 | Neutral olive oil in crural vein |
| February 14 | Rabbit | 110 | 30 | Neutral olive oil in jugular vein |
| February 15 | Dog | 105 | 20 | Neutral olive oil in jugular vein |
| February 28 | Dog | 140 | 40 | Neutral olive oil in jugular vein |
| March 25 | Cat | 108 | 38 | Neutral olive oil in jugular vein |
| March 26 | Cat | 90 | 35 | Neutral olive oil in jugular vein |
| The above experiment was repeated on the following dates: | | | | |
| *1919* | | | | |
| January 16 | Cat I | 180 | 30 | Neutral olive oil in jugular vein |
| January 16 | Cat II | 130 | 40 | Neutral olive oil in jugular vein |
| January 16 | Cat III | 105 | 55 | Neutral olive oil in jugular vein |
| *1922* | | | | |
| November 13 | Cat | 100 | 40 | Neutral olive oil in jugular vein |
| November 17 | Cat | 150 | 70 | Neutral olive oil in jugular vein |
| *1923* | | | | |
| January 24 | Cat | 110 | 52 | Neutral olive oil in jugular vein |

In table 1 are presented fourteen consecutive experiments made by the present writer. All but one of these experiments took a course similar to that illustrated in figures 1 and 2. The blood pressure fell steadily but not too rapidly after the injection into a large vein of about 1 cc. of neutral olive oil per kilo of body weight. The exception was a dog operated upon February 13, 1917.

*Experiment February 13, 1917.* Ten cubic centimetres of neutral olive oil were injected into the *crural* vein of a dog at 1:45 p.m. Twenty-five minutes later the carotid pressure was unchanged and 8 cc. more were injected. Again there was no fall, and at 2:31 p.m. 10 cc. of oil were injected. Artificial respiration was soon needed to supplement the dog's disordered breathing, and the carotid pressure first rose and then fell rapidly to 40 mm. Hg.

The crural vein was here employed. Had the oil been injected into the jugular vein and more time allowed for its action, the result would probably have been that seen in the other experiments.

Especial attention should be directed to the experiment of February 28, of which a full protocol is recorded in order that the reader may see the unmistakable symptom complex of shock.

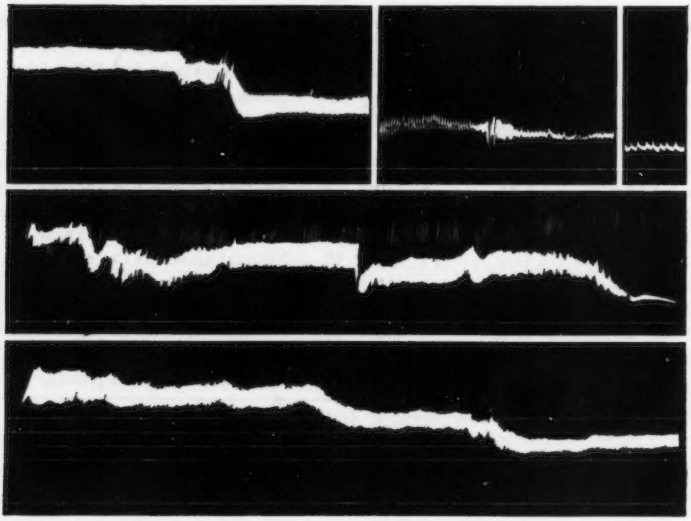

Fig. 1. Two-fifths the original size. Three experiments in which shock followed the injection of oil into the external jugular vein.

*February 5, 1917.* Carotid pressure in a cat. The first section of the curve begins at 11:40 a.m. and ends at 12 m. At 11:47, 10 cc. neutral cottonseed oil were injected. The pressure fell from 130 to 65 mm. Hg. At 12:11, when the middle section begins, froth poured out of the tracheal tube and artificial respiration was commenced. The middle section stops at 12:21. The third section extends from 12:27 to 12:30. The pressure then was 25 mm. Hg.

*February 6, 1917.* Middle curve. Carotid pressure in a rabbit during 32 minutes. Two minutes after the curve begins, 10 cc. thin cream were injected. Fourteen minutes later 4 cc. neutral olive oil were injected. In about 5 minutes more, artificial respiration was commenced. The pressure fell from 105 to 28 mm. Hg.

*February 7, 1917.* Lowest curve. Carotid pressure in a cat, during the first 33 minutes of the experiment. At the first mark on the atmospheric pressure line, 1 cc. neutral olive oil was injected; at the second mark, 2 cc., and at the third mark, 2 cc. The diastolic pressure now fell to 70 mm. (it had been 140 mm.). At the fourth mark, the cat's body was inclined, so that the feet were higher than the head.

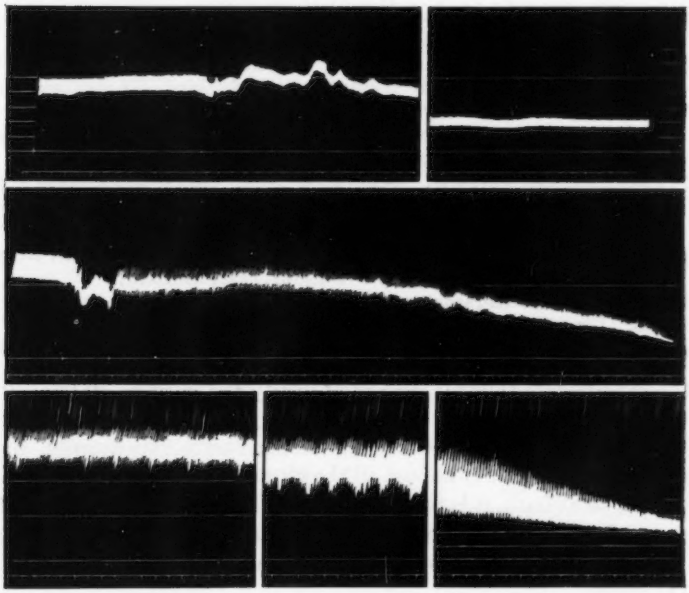

Fig. 2. One-half the original size. Three experiments in which shock followed the injection of oil into the external jugular vein.

*February 12, 1917.* Top curve. Carotid blood pressure in a cat. Membrane manometer. The graduation scale gives the rise of the lever for increments of 20 mm. Hg. Time in intervals of 30 seconds. An interval of 31 minutes separates the two portions of the curve. At the vertical mark in the atmospheric pressure line 3.5 cc. neutral olive oil were injected into the right jugular vein. This cat was curarized. The oscillations in the pressure curve are not due to muscular contractions; nor to the respiration; nor to the heart, the force of which remains fairly uniform; they are then probably caused by disturbances in the vasomotor centre. The diastolic pressure fell from 80 to 32 mm. Hg.

*February 14, 1917.* Middle curve. Carotid pressure in a rabbit. The atmospheric and the 100 mm. pressure lines are shown. Between the vertical marks on the atmospheric pressure line, 1 cc. of neutral olive oil was injected into the right jugular vein. At the second mark, artificial respiration was begun. The diastolic pressure fell from 110 to not over 30 mm.

*February 28, 1917.* Lowest curve. Carotid pressure in a dog weighing 6.5 kilos. At the left, pressure lines at 0,60 and 100 mm. Hg; at the right, 20, 40, 60, 80 and 100 mm. Time in intervals of 30 seconds. The experiment began at 10:05 a.m. with the injection of 15 cc. of neutral olive oil into the right external jugular vein. The blood pressure record began at 12:25. The first section here shown extends from 12:27 to 12:37 p.m. The middle section extends from 1:02 to 1:09 p.m.; the third section extends from 1:52 to 2:01 p.m. The diastolic arterial pressure fell from 140 to 40 mm. Protocol in text.

*Experiment February 28, 1917.* Shock produced by injecting 15 cc. neutral olive oil into the right external jugular vein of a dog weighing 6.5 kilos. The following notes are copied verbatim from the graphic record.

10:05 a.m. Cannula placed in the right external jugular vein. Fifteen cubic centimetres of neutral olive oil slowly injected. Wound sewed up.
11:15 a.m. Dog lies apparently but not really unconscious. Feces passed.
11:30 a.m. Heart 72 per minute; rather feeble; hesitating. Respiration 30; feeble. Rectal temperature 98.7°. Gums pale, paws and ears cold.
11:55 a.m. Heart 60; irregular. Respiration 22–26. Rectal temperature 97.8°.
12:25 p.m. Pulse 95. Respiration 20. Temperature 96°. Graphic record begun. Carotid diastolic pressure 140 mm. Hg (fig. 2, left curve).
1:14 p.m. Diastolic pressure 85 mm. Hg. Respiration less frequent; irregular; some edema of the lungs. Pulse 52; heart irregular.
1:30 p.m. Rectal temperature below 95°.
1:52 p.m. Diastolic pressure 60 mm. Hg.
2:00 p.m. Diastolic pressure 40 mm. Hg. Rectal temperature 93.2°. Death.

The experiments thus far presented justify the positive statement that shock may be caused by fat embolism. They do not disclose the mechanism by which this shock is caused. In the above experiments, the fat necessarily invaded all or almost all the structures in the body. To find a certain organ at fault, it is necessary first to limit the embolism to that one organ; and second, to embolize the remaining organs without producing the disorder. These ends are successfully gained in the observations next to be related.

It is natural, perhaps, that suspicion should fall first upon the lungs. The injected fat is carried directly to the lungs; respiration is always affected by the resulting embolism; and profuse acute pulmonary edema not seldom dramatically closes the scene. Indeed, many observers hold fast to the hypothesis that pulmonary embolism is the cause of this form of shock. It will presently be demonstrated by conclusive experiments that this hypothesis is erroneous. Since the pulmonary hypothesis must be rejected, the enquirer will turn to the vasomotor region: first, because it is the business of the bulbar vasomotor centre to conserve and to regulate the blood pressure, and a fall in blood pressure is the outstanding factor in shock; second, because much of the fat, in fat embolism, passes rapidly through the lungs into the systemic circulation and thus to the brain; third, because the plugs of fat have often been seen in the vessels of the brain; and fourth, because these emboli might easily interrupt the circulation in the vasomotor region, and it is known that the vasomotor cells are highly sensitive to variations in their blood supply. Such are the considerations which led to the experiments in which the vasomotor region alone is embolized.

A list of such experiments will be found in table 2. Neutral olive oil was injected into the brain end of one of the vertebral arteries. Since in the rabbit and in the cat this artery is inconveniently small, the injection

cannula was usually placed in the much larger subclavian artery, which was ligated on both sides of the origin of the vertebral. In the first two experiments (table 2), the quantity of oil injected was large (4 to 5 cc.). I did not then know how small a quantity would serve. One-tenth of one cubic centimetre per kilo of body weight has given a perfect result, as in the experiments of July 29 and July 30, 1918 (fig. 3). It must be borne in mind that a considerable part of this small quantity remains in the stretch of artery between the point of injection and the capillaries of the bulb. Moreover, muscular branches come off from the subclavian often so close to the origin of the vertebral as necessarily to be left within the pouch

TABLE 2

*Injection of oil into the brain end of the vertebral artery*

| DATE | ANIMAL | DIASTOLIC CAROTID PRESSURE | | NATURE OF EMBOLI |
|---|---|---|---|---|
| | | Before injection | After injection | |
| *1918* | | *mm.* Hg | *mm.* Hg | |
| April 15 | Cat | 80 | 40 | 4.5 cc. neutral olive oil into a pouch formed by ligating the subclavian artery on both sides of the origin of the vertebral artery |
| May 3 | Cat | 110 | 60 | 5 cc., as above |
| July 26 | Rabbit I | 118 | 42 | 0.5 cc. in vertebral |
| July 26 | Rabbit II | 135 | 30 | 0.5 cc. in vertebral |
| July 26 | Cat | 118 | 60 | 0.5 cc. in vertebral |
| July 29 | Rabbit | 160 | 40 | 0.2 cc. in vertebral. See fig. 3 |
| July 30 | Rabbit | 130 | 43 | 0.2 cc. in vertebral. See fig. 3 |
| The above experiment was repeated on the following dates: | | | | |
| *1923* | | | | |
| December 26 | Rabbit | 110 | 38 | 0.3 in vertebral |
| December 29 | Rabbit | 135 | 35 | 0.6 in vertebral |

formed by the two ligatures placed upon the subclavian. In such a case, a part of the 0.2 cc. of oil injected must find its way into those muscular branches. The quantity of oil which remains for the bulbar vasomotor region after these losses is therefore very small. Not all of this remainder is caught in the bulbar capillaries; some of it passes through the bulb into the veins and thus back into the pulmonary circulation. It is inconceivable that so minute a portion should affect the lungs. Another portion of the 0.2 cc. injected might, academically speaking, find its way into the circle of Willis and thus into parts of the brain anterior to the bulb. This portion need not detain us; the whole of the brain anterior to the bulb may be separated from the bulbar vasomotor region without lowering the

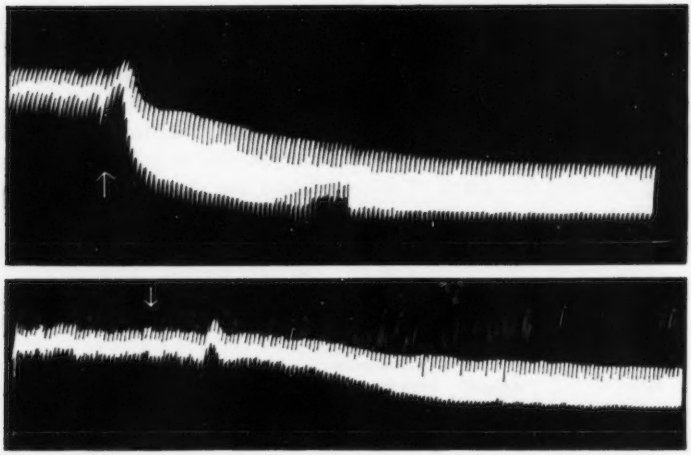

Fig. 3. *Experiment July 29, 1918.* Upper curve. The original size. A rabbit weighing 2 kilos was lightly curarized and artificial respiration was begun. The carotid diastolic blood pressure was 160 mm. Hg. The subclavian artery was ligated at its origin from the aorta and also at a point beyond the origin of the vertebral artery. The internal mammary branch was ligated, but two muscle branches were left open. By means of a cannula in the subclavian, one-fifth cubic centimetre of neutral olive oil was injected (see arrow). Part entered the muscle branches and part the vertebral. The blood pressure fell to 40 mm. Hg.

*Experiment July 30, 1918.* Lower curve. Two-thirds the original size. Carotid pressure in lightly curarized rabbit weighing 2 kilos. Preparation as on July 29. At the arrow, 0.2 cc. neutral olive oil was injected into the subclavian; a portion passed into the vertebral artery and thus to the bulb. The diastolic pressure fell from 130 to 43 mm. Hg.

blood pressure. Since these escaping portions are negligible, the conclusion is justified that shock may be produced by fat embolism of the vasomotor region alone.

### V. THE RELATION OF THE CAROTID AND VERTEBRAL ARTERIES TO FAT EMBOLISM OF THE VASOMOTOR CENTRE

If fat embolism of the vasomotor centre is a cause of shock, it might be supposed that shock would follow the injection of oil through the cerebral end of the carotid artery. It often does not. This frequent failure must be explained.

It might be supposed that the circle of Willis is an open road by which oil passing through the carotid would easily reach the nerve centres in the bulb. The circle of Willis is undoubtedly a generous anastomosis—

from an anatomical point of view. This large anastomosis is the basis of the accepted opinion that the pressure in the circle of Willis is maintained by four contributing arteries—the two carotids and the two vertebrals—an opinion not borne out by experimental evidence. On May 6, 1919, and May 8, 1924, a cannula was placed in the brain end of one carotid artery in the cat as near the circle of Willis as possible. The blood pressure in this brain end of the carotid may be taken as approximately the pressure in the circle of Willis itself. It varied from 43 to 65 mm. Hg. The experiments were in two sets. In the first set, both vertebrals were left open and both carotids were closed. The results were as follows.

| DATE | PRESSURE IN THE CIRCLE OF WILLIS | PRESSURE ON CLOSING SECOND CAROTID; BOTH VERTEBRALS STILL OPEN |
|---|---|---|
| *1919* | *mm.* Hg | *mm.* Hg |
| May 6 | 65 | 42 |
| May 6 | 60 | 38 |
| *1924* | | |
| May 8 | 47 | 10 |
| May 8 | 50 | 10 |
| May 8 | 43 | 7 |
| May 8 | 50 | 9 |
| May 8 | 53 | 10 |

Closing both carotids caused a great fall in the pressure in the circle of Willis.

In the second set of experiments, the second carotid artery was left open and both vertebral arteries were closed. The pressure in the circle of Willis remained unchanged in every instance.

It is evident from these observations that the blood pressures in the circle of Willis are not so simple as the anatomical relations would lead us to believe. The anatomical relation is an unsafe guide in deciding upon the path which an embolus shall take. The direction taken by a drop of oil entering the circle of Willis will not finally depend upon anatomical but upon physiological considerations. It will depend on the blood pressure in the several branches of the circle, and on the peripheral resistance in their areas.

It has already been shown in table 1 that the injection of 1 cc. of oil per kilo through the external jugular vein causes shock. In those experiments one carotid and both vertebral arteries were open. Very different is the result when the carotids are open but the vertebrals closed. In 1919, 3.5 to 4.0 cc. of oil were injected into the jugular vein of each of five cats in which the vertebrals were closed. The diastolic crural pressures before and after injection were as follows:

| DATE | DIASTOLIC BLOOD PRESSURE BEFORE INJECTION | DIASTOLIC BLOOD PRESSURE AFTER INJECTION |
|---|---|---|
| *1919* | *mm.* Hg | *mm.* Hg |
| January 28 | 100 | 95 |
| February 4 | 130 | 125 |
| February 5 | 85 | 80 |
| February 6 | 110 | 105 |
| February 12 | 120 | 115 |

Closure of the vertebral arteries kept the vasomotor region free of emboil although both carotid arteries were open.

Exactly opposite is the result when both carotids are closed and both vertebrals are open.

| DATE | DIASTOLIC BLOOD PRESSURE BEFORE INJECTION | DIASTOLIC BLOOD PRESSURE AFTER INJECTION |
|---|---|---|
| *1919* | *mm.* Hg | *mm.* Hg |
| January 24 | 110 | 52 |
| January 27 | 130 | 100 |
| January 28 | 110 | 40 |
| February 14 | 98 | 50 |
| February 25 (1) | 160 | 70 |
| February 25 (2) | 165 | 65 |
| March 11 | 90 | 47 |

In all but one of these observations, the diastolic pressure fell profoundly.

Examine now the results of injecting 0.5 cc. of the oil into the brain end of the carotid artery. The experiments are in two series. In the first, one carotid artery is closed and both vertebrals are open, throughout the experiment.

| DATE | DIASTOLIC BLOOD PRESSURE BEFORE INJECTION | DIASTOLIC BLOOD PRESSURE AFTER INJECTION |
|---|---|---|
| *1918* | *mm.* Hg | *mm.* Hg |
| July 2 | 120 | 105 |
| July 15 | 120 | 80 |
| *1919* | | |
| March 1 | 140 | 100 |
| March 14 | 115 | 35 |
| March 17 | 100 | 80 |

Some fall of pressure takes place in each of the four experiments, but in one only is the fall pronounced. In the experiment of July 15 (lower half of fig. 4) the lost pressure was soon regained. Obviously, closing one carotid impairs the balance in the circle of Willis. If our reasoning is correct, closing both carotids should have a greater effect—the balance should be not only impaired, but so to say destroyed. In the next series

of experiments, therefore, both carotid arteries were closed and both vertebrals were left open.

| DATE | DIASTOLIC BLOOD PRESSURE BEFORE INJECTION | DIASTOLIC BLOOD PRESSURE AFTER INJECTION |
|---|---|---|
| *1918* | *mm.* Hg | *mm.* Hg |
| July 3 | 120 | 40 |
| July 12 | 150 | 60 |
| July 17 | 82 | 45 |
| July 22 | 100 | 60 |
| July 24 | 98 | 42 |
| *1919* | | |
| March 13 | 125 | 60 |
| April 7 | 120 | 40 |
| April 9 | 140 | 70 |

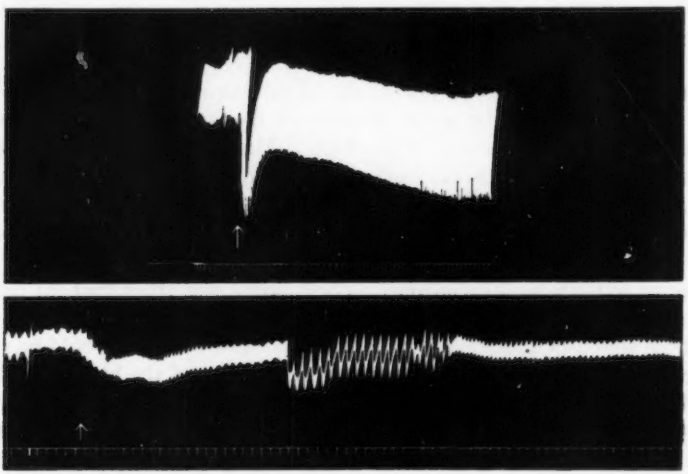

Fig. 4

*Experiment, July 22, 1918.* Upper curve. The original size. The carotid pressure in a cat. Both carotids closed throughout the experiment. At the arrow, 0.5 cc. neutral olive oil was injected into the cerebral end of one carotid. Artificial respiration was required ten minutes later. The diastolic arterial pressure fell from 100 mm. Hg, before the injection, to 60 mm. at the end of the curve. The experiment lasted one hour.

*Experiment July 15, 1918.* Lower curve. Four-fifths the original size. The carotid blood pressure in a cat. The second carotid was free throughout the experiment. Five times injections of 0.5 cc. neutral olive oil were made into the cerebral end of the first carotid. The section of the curve here given shows the effect of the third injection. The arrow marks the point at which the oil (0.5 cc.) began to enter the carotid. There was an immediate temporary fall in the blood pressure. In about 90 seconds the breathing was so affected that artificial respiration was advisable. The difficult breathing soon became normal. The diastolic arterial pressure at the beginning and end of the curve is almost the same.

The great fall which followed the injection of 0.5 cc. of oil in the brain end of the carotid, both carotids being closed, is the more striking because in the last six experiments of the above series, the closure of the second carotid was during the injection only—the clamp was removed a few moments after the injection. A carotid pressure curve from the experiment of July 22, 1918, is given in the upper part of figure 4, and a photomicrograph[8] from the experiment of July 3, 1918, is shown in figure 5.

Enough has now been said to show how great is the difference between the carotid and the vertebral artery in their relation to the nutrition of the bulb. Whether oil injected into the veins or directly into the general circulation will or will not cause shock depends upon the path taken by the oil. If the oil causes an adequate anemia of the vasomotor area, shock will take place. If the oil takes another course, or if the quantity entering vessels going to the vasomotor region is insufficient, shock will not take place.

### VI. FAT EMBOLISM OF THE LUNGS IS NOT A CAUSE OF SHOCK

Since in clinical cases the fat in the blood can reach the bulb only by first passing through the lungs, pulmonary fat embolism will always be present in clinical embolism of the bulb. Many observers contend that fat embolism of the lungs is in itself a cause of shock. Were this hypothesis true, it would be impossible to prove that shock is ever due to bulbar fat embolism; it could always be referred in whole or in part to the accompanying, clinically inseparable, embolism of the lung. Fortunately, this beguiling hypothesis is erroneous. These pages will show that fat embolism of the lungs is not a cause of shock.

Those who urge pulmonary embolism as a cause of shock would have us believe that the arterial pressure falls because some of the blood vessels in the lungs are stopped. Yet, in pneumonia, the arterial pressure does not usually fall, although in pneumonia more vessels may be closed than in fat embolism. The "factor of safety" in the lungs is so large that even the sudden removal of whole lobes does not much affect the systemic blood pressure.[9] Thus in the experiment of March 29, 1918, figure 6, the dias-

[8] The experiment of July 3, 1918, was by Porter and Emerson. The photomicrograph reproduced in figure 5 was made by Dr. S. B. Wolbach, Shattuck Professor of Pathological Anatomy in Harvard University, to whom I am much indebted.

[9] Compare Scriba, Deutsch. Zeitschr. f. Chirurgie, September, 1879, xii, 205.

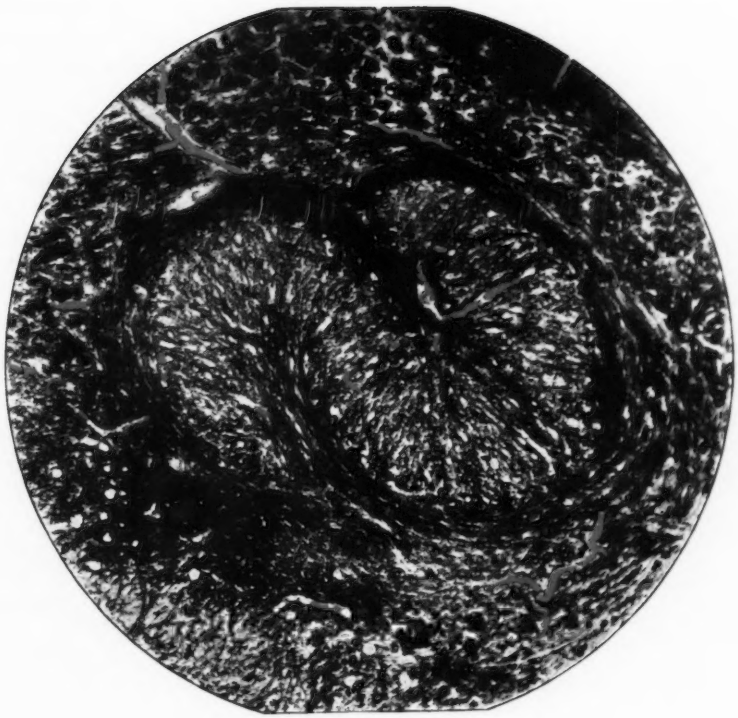

FIGURE 5

Part of a transverse section through the bulb at a little less than two-thirds the distance from the calamus scriptorius to the corpora quadrigemina. The fat emboli in the blood vessels were stained with Scharlach R. From an experiment on a cat July 3, 1918, in which neutral olive oil was injected into the cerebral end of one carotid artery. The other carotid was closed. Both vertebral arteries were open. The diastolic arterial pressure fell from 120 mm. to 40 mm. Hg. Fat embolism was frequent throughout the vasomotor region.

tolic carotid pressure was recorded in a cat, and at the arrow the root of the right lung was clamped so as to shut that lung completely from the circulation. After some slight oscillation, the carotid pressure resumed the normal level. A priori, then, we may be sure that the fall of pressure

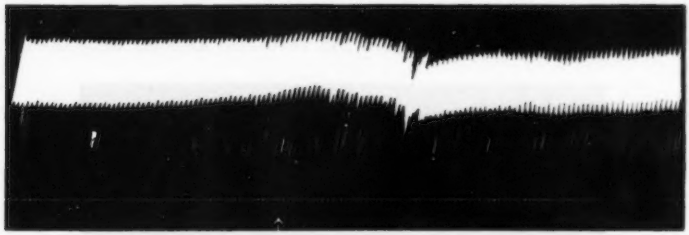

Fig. 6. *Experiment March 29, 1918.* **Four-fifths the original size.** Carotid pressure in a cat. Open chest. Artificial respiration. At the arrow, the root of the right lung was clamped so as to shut that lung completely from the pulmonary circulation. The arterial pressure in the aortic circulation was but little changed.

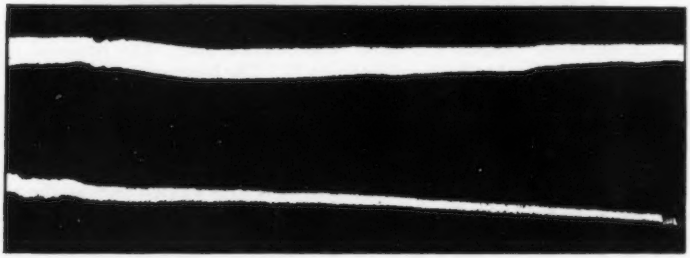

Fig. 7. *Experiment March 28, 1918.* One-half the original size. Carotid pressure in a cat. Time at intervals of 5 seconds. Artificial respiration. Both vertebrals closed. At 12:10 p.m. closed second carotid (the first carotid was already closed by the cannula). The diastolic arterial pressure now fell from 80 to 67 mm. Hg. At 12:27 p.m. the upper curve in this figure begins. At 12:30, 3.7 cc. neutral olive oil were injected into the right external jugular vein. This oil caused a great pulmonary embolism, but the blood pressure did not fall, because the oil did not reach the brain, or reached it in quantities too small to affect the vasomotor region. The *lower curve*, from the same experiment, begins at 12:58 p.m. The second carotid artery had been released from its clamp eight minutes previously. At 1 p.m., 3.5 cc. olive oil were injected into the jugular vein. This oil could now reach the brain, by passing through the lungs, and the blood pressure fell from 70 mm. to 27 mm. Hg.

observed in fat embolism is not due to obstruction of the pulmonary circulation, since obstruction as great or greater observed in pneumonia or produced experimentally has no such effect.

Experiments have already been cited in which shock was produced by emboli limited to the vasomotor region, and in which the lungs were free of fat.

Again, the reader is reminded of the experiments on pages 288 to 289, in which 1 cc. of oil per kilo is injected into the jugular vein. In series A, the vertebral arteries were closed and the carotids open; in series B, the carotid arteries were closed and the vertebral arteries were open. In

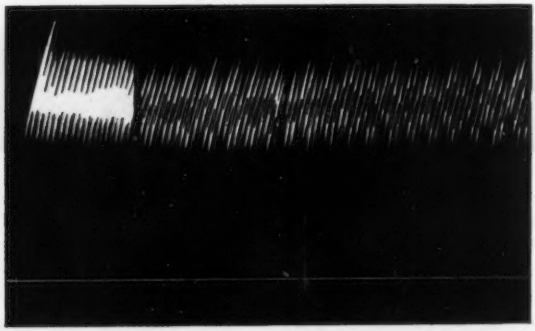

Fig. 8. *Experiment August 11, 1923.* The original size. The brain of cat A (weight 3288 grams) was fed by blood from cat B. The heart end of the left carotid of cat B was connected with the brain end of the left carotid of cat A. The blood flowing from B to the brain of A was returned to B by connecting the heart end of the left carotid of A with the brain end of the left carotid of B. Each animal received through an external jugular vein 1 decigram of heparin per kilo of body weight, to prevent coagulation. Both subclavian arteries of A were ligated to close the vertebral arteries. The right carotid of A was clamped. The blood pressure was written by the femoral artery. Rate of drum, 11 mm. per minute. The experiment began at 12:40 p.m. and was stopped at 1:40 p.m. The first section of the curve shows the first two minutes of the experiment; the second section shows the last three minutes. The interval is 55 minutes. At 12:44, at 12:49 and at 12:54, respectively, 1 cc. neutral olive oil was injected into the right external jugular vein of A. At 1:24 foam poured out of the tracheal opening. In spite of acute edema of the lungs the blood pressure was as high at the end of the experiment as at the beginning. This was also the case with cat B.

series A, shock did not occur, because the oil was excluded from the vasomotor region by the closure of the vertebral arteries; in series B, shock did occur, because these arteries were open and thus the path to the vasomotor region was open. Yet the lungs were embolized equally in both series. Obviously, the state of the lungs being identical in both series, the difference in the result must be due to a factor outside the lungs. The experiments point clearly to embolism of the vasomotor region as the cause of the shock observed in series B, in which the vertebral arteries were open. Figure 7 is a witness to the truth of these observations.

Finally, it is possible, by means of a circulation crossed between two animals, to separate the circulation of the brain from that of the body, and to embolize either at will. The experiment of August 11, 1923 (fig. 8), is an example of this procedure.

*Experiment August 11, 1923.* The brain of cat A was supplied with blood by a circulation crossed with cat B. At 12:40 p.m. the diastolic crural pressure of cat A was 107 mm. Hg. At 12:44, 12:49 and 12:54 p.m., respectively, 1 cc. of neutral olive oil was injected into the right external jugular vein of A. The brain of cat A was open only to the blood coming from cat B. The systemic blood pressure of cat A did not fall, though the lungs of A were so filled with fat that the resulting pulmonary edema caused foam to pour from the trachea. At 1:40 p.m. the blood pressure in A was as high as at 12:40 p.m., before the oil had been injected.

Identical results were obtained with other cats.

Under the conditions of the experiment of August 11, 1923, the oil first entered the lungs of cat A. The greater part was there retained. A portion passed the pulmonary capillaries and was carried to the left auricle and ventricle of A. A smaller portion was driven thence through the cardiac end of the carotid of A into the brain end of the carotid of B. Thence it entered the brain of B. All or nearly all must have stopped in the cerebral capillaries of B. Any portion not arrested by this second net passed into the veins of B and so to the right heart of B. From there it found its way to the pulmonary capillaries of B. It is probable that only a minute amount could finally have escaped this third capillary filtration. This ultimate possible portion could have passed through the carotid arteries of A to the brain of A. Certain it is that the amount which in the end might have reached the vasomotor region of A was too small to have the slightest effect upon the blood pressure, as shown by figure 8. The result of a careful microscopic examination of the vasomotor region of A supports this statement. The bulb of A was fixed in formalin, frozen, and cut into thin serial sections beginning near the calamus scriptorius and ending near the corpora quadrigemina. Every tenth section was stained with Scharlach R and counter-stained with hematoxylin. In one of these section a single minute particle of fat was found—all the other sections were free.

These several experimental processes, each so different from the others, lead to the same unequivocal conclusion—that pulmonary embolism is not a cause of shock; pulmonary embolism need not lower the blood pressure, even when pushed to a violent acute edema.

## VII. SHOCK NOT DUE TO EXHAUSTION OF THE VASOMOTOR CENTRE.

The hypothesis that shock is caused by the exhaustion of the vasomotor centre is said to have been originally set forth by Mitchell, Morehouse

and Keen[10] sixty years ago. "Exhaustion," strictly defined, would mean the failure of the vasomotor centre from overstimulation. It is necessary to consider this idea here, because the wounds which are followed by fat embolism shock undoubtedly stimulate afferent nerves, and it might be thought that this stimulation, by fatiguing the vasomotor centre, would completely or at least partially account for the shock ascribed by me to fat embolism.

Efforts to fatigue the vasomotor centre by the persistent stimulation of afferent nerves were made by me in 1905, 1907 and 1909. The following abbreviated protocols are examples of these experiments.

*Experiment June 27, 1905.* The spinal nerves in the lumbar region of a cat were stimulated for three hours and fifty-five minutes, beginning at 10:50 a.m. At 2 p.m. the blood pressure had fallen but 20 mm. Hg.

*Experiment September 6, 1907.* At intervals of one or two minutes, strong induction currents were passed for thirty seconds through the central end of the sciatic nerve of a cat. This continued from 11:30 a.m. to 1:30 p.m. At the beginning, the blood pressure was 80 mm.; on stimulation of the sciatic nerve, it rose to 105 mm.; two hours later the blood pressure was 60 mm.; on stimulation of the sciatic, it rose to 113 mm.[11]

*Experiment February 9, 1909.* The depressor nerve of a rabbit was stimulated at frequent intervals during eight hours with an inductorium set at 1000 Kronecker units. At 10 a.m., 2 p.m. and 5:40 p.m., the resultant absolute fall in blood pressure was 35, 41, and 42 mm., respectively.[12]

I have not been able to exhaust the vasomotor centre by any stimulation, however prolonged. The exhaustion hypothesis, indeed, does small credit to the wonderful mechanism by which the distribution of the blood is controlled. Sensitive this mechanism is, but it is also highly resistant. It is very possible that the vasomotor centre cannot be fatigued. It is certain that it cannot be fatigued by the prolonged stimulation of afferent nerves.

The literature of the past sixty years shows that the supporters of the exhaustion hypothesis have in mind something more than the fatigue of the vasomotor centre by overstimulation. "Exhaustion," to them, seems to mean simply the failure of the vasomotor centre. But we shall now show that the vasomotor centre does not fail in shock, except in that which follows fat embolism of the bulb and concussion of the bulbar cells. The experiments now to be presented will divide shock into two categories: in the one, the vasomotor centre is normal; in the other, it is directly attacked. I cite first the experiments of 1903, in which the vasomotor

[10] Mitchell, Morehouse and Keen, Circular no. 6, Surgeon-General's Office, March 10, 1864.

[11] Porter, Marks and Swift, This Journal, 1907, xx, 444–449.

[12] Porter, This Journal, 1910, xxvii, 282.

centre gave a normal response to the stimulation of the depressor nerve in shock produced by injury of the abdominal viscera. An example follows.

*Experiment September 24, 1903.*[13]

9:00 a.m. Rabbit anesthetized with ether.

9:15 a.m. Carotid blood pressure 67 mm. Hg. Rectal temperature 38°C. On stimulation of the depressor nerve the blood pressure fell to 36 mm., a fall of 46 per cent.

9:20 to 9:30 a.m. Applied nitric acid to exposed intestines. The blood pressure at first rose slightly and then sank slowly; the rectal temperature also sank steadily. Shock soon progressed so far that the anesthetic was no longer necessary.

3:25 p.m. Rectal temperature 26°C.

5:16 p.m. Rectal temperature 25°C.

The effect of depressor stimulation during the eight hours of the experiment was as follows.

| HOUR | BLOOD PRESSURE BEFORE STIMULATION OF DEPRESSOR | BLOOD PRESSURE DURING STIMULATION OF DEPRESSOR | ABSOLUTE FALL | PERCENTILE FALL |
|---|---|---|---|---|
| | *mm.* Hg | *mm.* Hg | *mm.* Hg | *per cent* |
| 9:15 a.m. | 67 | 36 | 31 | 46 |
| 3:25 p.m. | 53 | 30 | 23 | 43 |
| 3:30 p.m. | 53 | 30 | 23 | 43 |
| 4:50 p.m. | 40 | 22 | 18 | 45 |
| 5:16 p.m. | 35 | 23 | 12 | 34 |

Both the absolute and the percentile fall of blood pressure were normal in this case many hours after shock was present. Consider that the abdomen was opened and the intestines were painted with nitric acid at 9:15 a.m., that the animal soon developed symptoms of shock, that ether was presently unnecessary, that the temperature fell steadily, and that the animal lay six hours in this condition before the record of 3:30 p.m. was taken. The rectal temperature had then fallen 12 degrees Centigrade. Yet at 3:30 p.m. the absolute and the percentile fall in pressure on stimulating the depressor nerve were still equal to those obtained before shock was induced. An hour and twenty minutes later, over eight hours after the intestines were exposed, death was near; only then did the vasomotor apparatus begin to fail.

In other experiments of that year, 1903, the blood pressure, which had fallen in shock, was temporarily raised to normal by the injection of saline solution in the jugular vein, and the depressor immediately stimulated. In such cases the blood pressure fell as many millimetres as it had before shock set in.

[13] Porter and Quinby, This Journal, 1904, x, Proc. Amer. Physiol. Soc., 1904, Feb. 1, pp. xii and xiii. Also Porter and Quinby, Boston Med. and Surg. Journ., 1903, cxix, 455. See also this Journal, 1908, xx, 500–505.

The reader will have noted that in these experiments stress is laid upon the percentile method of reckoning the blood pressure, there used for the first time. To measure the changes in the arterial pressure is naturally of great importance to physiologists. A new procedure which claims to be, under certain conditions, the only method of measuring blood pressure correctly, should be received with skepticism. No doubt for this reason an occasional critic has looked somewhat coldly on the experiments of 1903. But these critics overlooked the fact that in the experiments of 1903 the absolute as well as the percentile fall of blood pressure proved that the vasomotor centre was normal after shock had set in. Hence the proof that the vasomotor cells may be normal in shock would remain even if the percentile measurements were withdrawn. But the percentile measurements are nevertheless highly valuable, since there are times when they alone give a correct measure of the activity of the reflex vasomotor arc, as in the first experiment in which the percentile method was used—the experiment of September 24, 1903, just cited.

The protocol of September 24, 1903, shows that at 9:15 a.m. stimulation of the depressor gave an absolute fall of 31 mm. Hg. The intestines were then exposed and painted with nitric acid. Shock rapidly came on. Six hours later, at 3:30 p.m., stimulation of the depressor gave an absolute fall of 30 mm. Hg., practically that secured before shock was induced. The vasomotor cells were then normal although shock had been present for several hours. But at 4:50 p.m., the absolute fall on depressor stimulation was 22 mm. If the absolute fall is the correct measure, the vasomotor cells were now partly exhausted; if, on the contrary, the percentile fall is the correct measure, the vasomotor cells were still normal, since 31 mm. is 46 per cent of the initial blood pressure at 9:15 a.m. and 22 mm. is 45 per cent of the blood pressure at 4:50 p.m. This question, so serious in its wide implications, was answered by other observations in the series of which the experiment of September 24, 1903, is an example. Shock was again brought on, and the blood pressure sank. Stimulation of the depressor nerve at length gave less than the normal fall. Does this mean that the vasomotor centre was then impaired? By no means, for when the blood pressure was temporarily raised to normal by injections of saline solution, the same stimulation of the depressor gave again the normal absolute fall, i.e., the blood pressure fell as many millimetres as it had before shock set in. The experiments of 1903 proved, therefore, that the percentile change in blood pressure is a true measure of the condition of the vasomotor centre.

It is obvious that the blood pressure, or state of distention of the arteries, is the tool with which the vasomotor centre works. In the experiment of September 24, 1903, the blood pressure at the beginning of stimulation was 67 mm. and excitation of the depressor caused it to fall 31 mm. Hg. When

the blood pressure, seven hours later, was only 40 mm., the tool was changed, and an absolute fall of 31 mm. was impossible. The fault lay with the flaccid arteries, not with the depressor nerve or the vasomotor cells. When, in such experiments, the arteries are given their former distention by the injection of normal saline solution, the tool is restored, and the blood pressure again gives a normal absolute fall on depressor stimulation, in spite of the profound shock.[14]

Of course, there must be an end even to the powers of the vasomotor centre. In the rabbit of September 24, 1903, the vasomotor centre began to fail seven hours or more after shock was induced; at 5:16 p.m. depressor stimulation caused a fall of 34 instead of 45 per cent. The rectal temperature was then 25°C. Death was at hand.

In 1914, I again pointed out that a correct choice between the absolute and the percentile method of reckoning is of the highest importance, because this choice determines quantitatively the normal reflex and such a reflex is the only evidence that the vasomotor apparatus is normal. In the research of 1914,[15] the reflex change in blood pressure on stimulating the central end of the sciatic, brachial and depressor nerves in cats and rabbits was measured when the initial blood pressure was at different levels. The results with the sciatic nerves were as follows.

| INITIAL BLOOD PRESSURE | ABSOLUTE RISE | PERCENTILE RISE |
|---|---|---|
| *mm.* Hg | *mm.* Hg | *per cent* |
| 70 to 89 | 53 | 73 |
| 50 to 69 | 48 | 74 |
| 30 to 49 | 25 | 70 |
| 20 to 29 | 18 | 73 |

The absolute rise here shows a large progressive failure of the vasomotor arc; whereas the percentile change shows no failure. The absolute rise is here a false, and the percentile rise a true criterion, for the vasomotor apparatus was actually normal throughout the measurements. When the low initial pressure was raised to normal by injecting defibrinated blood or normal saline solution, stimulation of the sciatic gave the usual normal rise.

The facts here set forth have been confirmed by the valuable experiments of Seelig and Lyon.[16] These observers used dogs. Both vagi were severed. Stimulations of the central end of one of the vagi with an induced current

[14] For percentile method, see also this Journal, xx, 1907, 402; Ibid., 1908, xxi, 461; 1908, xxiii, 132 *et seq.*; and, especially, Ibid., 1914, xxxiii, 373–377.

[15] This Journal, 1914, xxxiii, 373–377.

[16] Seelig and Lyon, Surgery, Gynec. and Obstetrics, 1910, xi, 146–152. See also Journ. Amer. Med. Assoc., Jan. 2, 1909, p. 45.

gave a fairly constant rise of blood pressure. After the constancy of this rise was determined, shock was induced. Repeated stimulations demonstrated that the vasomotor centre was not exhausted. Moreover, it was found that for each dog used there was a definite strength of current necessary to awaken a vasomotor response. The weakest stimulation which succeeded in calling forth a pressor effect was called the minimal stimulation. A further increase in the strength of the current invariably caused a more pronounced pressor effect, which was termed the maximal stimulation. Within these limits, the reflex rise was proportional to the strength of the stimulus. Thus were secured percentile variations in rise of pressure dependent upon the tonicity of the vasomotor centre, and the "authors were able, by repeating these maximal and minimal stimulations, to show

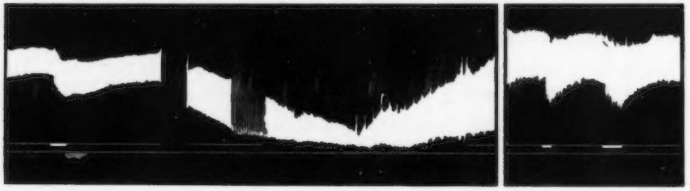

Fig. 9. *Experiment January 2, 1924.* Sixteen-seventeenths the original size. To show that the vasomotor centre is normal in abdominal shock. Carotid blood pressure in a rabbit. At 11:32 a.m. the left depressor nerve was stimulated. The diastolic pressure fell from 70 mm. to 50 mm. Hg, 29 per cent. At the break in the curve, 11:33 to 11:41 a.m., the intestines were drawn out of the abdomen. The diastolic pressure fell rapidly, and artificial respiration became necessary. At 12:20 p.m., the diastolic pressure was raised from 38 mm. to 70 mm. Hg by injections of normal saline solution mixed with adrenalin. Stimulation of the depressor now caused the diastolic pressure to fall from 70 mm. to 50 mm., 29 per cent, and from 70 to 48 mm., 31 per cent. The absolute fall in the three observations was 20, 20 and 22 mm. Hg, respectively.

that even in the profoundest shock, with the blood pressure alarmingly low, varying strength in the stimuli called forth corresponding rises in pressure. Furthermore, the minimal stimulation necessary to call forth a pressor effect remained practically constant both before and after the induction of profound shock, thus suggesting the fact that even in deep shock the tone of the vasomotor centre was not depressed, as regards response to electrical stimulation."

Thus the work of Seelig and Lyon is entirely in accord with the conclusions reached by me in 1903 and 1914.

In 1924 I once more returned to this subject—the condition of the vasomotor centre in shock—with results illustrated by figure 9 and the accompanying protocol.

*Experiment January 2, 1924.* The carotid pressure was recorded in a rabbit, beginning at 11:30 a.m. At 11:32 a.m. the left depressor nerve was stimulated, causing the diastolic pressure to fall from 70 mm. to 50 mm. Hg. The abdomen was then opened and the intestines were drawn out. The pressure fell steadily to 38 mm. and the respiration became very feeble. Artificial respiration and injections of warm saline solution containing small quantities of adrenalin raised the pressure again for short periods. From time to time, during these periods, the depressor was again stimulated.

| HOUR | DIASTOLIC PRESSURE | | ABSOLUTE FALL | PERCENTILE FALL |
|---|---|---|---|---|
| | Before stimulation of depressor | After stimulation of depressor | | |
| | *mm.* Hg | *mm.* Hg | *mm.* Hg | *per cent* |
| 11:32 a.m. | 70 | 50 | 20 | 29 |
| 11:55 | 70 | 48 | 22 | 31 |
| 12:03 p.m. | 80 | 57 | 23 | 29 |
| 12:09 | 70 | 50 | 20 | 29 |
| 12:20 | 70 | 50 | 20 | 29 |
| 12:21 | 70 | 50 | 20 | 29 |
| 12:22 | 70 | 48 | 22 | 31 |
| 12:23 | 65 | 47 | 18 | 28 |

The stimulations at 11:32 a.m., 12:21 p.m., and 12:22 p.m. are given in figure 9.

The observations of 1924, of which the protocol of January 2 is an example, agree with those of 1903 and 1914. The vasomotor cells are not injured in shock produced by violence to the abdominal viscera, still the most usual method of causing shock. Shock in which the vasomotor centre is normal is a demonstrated fact.

The condition of the vasomotor centre in fat embolism shock is precisely opposite. In section VIII, it will be shown that in fat embolism shock the vasomotor centre gave no reply whatever to stimulation of the depressor nerve.

Thus the problem in hand is answered; sound experimental evidence proves the existence of two main orders of shock; in one of which the vasomotor centre is normal, whereas in the other the vasomotor centre is damaged.

## VIII. VARIOUS TYPES OF SHOCK

Shock is a symptom complex—the final common scene in not a few pathological states. The new disease—fat embolism of the vasomotor centre—will be more exactly apprehended, if the reader will give some thought to the varied pathological conditions among which it takes a place. To this end, the history of the subject before and after the year 1917 should be borne in mind. Before 1917, it was not understood that shock is a stage in widely different disorders which have little else in com-

mon. Before that date, it was supposed that shock is itself a disease, brought on by abdominal injuries, fractures, etc., but, when once induced, a pathological state *per se*. In my Harvey lecture of 1917, various types of shock were definitely separated.[17] These several types were as follows: the hydrostatic lowering of the blood pressure, vibration injuries, wounds of the vasomotor apparatus, and hemorrhage. To these in 1918 was added a new type, namely, fat embolism of the vasomotor centre. Thus the modern history of shock may be said to begin with the idea that shock is a single disease; in 1864, exhaustion of the vasomotor centre is put forward as the cause of this disease; in 1903, exhaustion is overthrown; in 1917, the idea of a single cause gives way to the conception that shock is a symptom complex common to various and quite distinct pathological states; and finally, in 1918, a new disease is added to the other disorders which have shock as a common symptom.

It is now possible to attempt the perilous task of a general classification.

There are two orders of shock. In the first, the symptom complex is produced without injury to the vasomotor centre. In the second, the vasomotor cells are directly attacked.

The first order is illustrated by hemorrhage; when sufficient blood is lost, the general blood pressure falls and the symptoms of shock appear. Another example of the first order is found in dilatation of the abdominal vessels (without injury to the splanchnic nerves); so much blood then flows into these widened vessels that the general blood pressure is again lowered and the clinical picture of shock appears. A third example of the first order is seen in those injuries which sever important vasomotor paths, as when the splanchnic nerves are damaged. If, in all three of these disorders, the blood pressure is brought to normal and the state of the vasomotor centre tested quantitatively by the stimulation of afferent nerves, the bulbar vasomotor cells will be found still normal. The second order, in which the vasomotor cells are from the beginning directly affected, includes three very different pathological states; *a*, vibration injuries, i.e., concussion of the vasomotor cells, from blows on the skull; *b*, fat embolism of the bulbar vessels, and *c*, the action of poisons on the vasomotor cells. We must now briefly examine these various types of shock.

*Hemorrhage; and dilatation of the abdominal vessels.* The relation of external hemorrhage to shock has been clouded by misconceptions which spring mainly from ignorance of the relation between shock and what I have termed the critical level of the blood pressure. The critical level of blood pressure is that point below which the blood pressure will not usually

[17] Harvey Lectures, 1917–1919, Lippincott, 1920, pp. 21–43. Compare Harvey Lectures for 1906–07, Lippincott, 1908, pp. 98–116. First printed in the Boston Med. and Surg. Journ., Jan. 16, 1908, clviii, 73–79.

rise without assistance.[18] Measured by the Vaquez instruments used by me in France the normal diastolic arterial pressure in man was 97, and the critical level about 60, i.e., between two-thirds and three-fifths of the normal. Since the normal diastolic reading varies slightly with the instrument employed, it is probable that the critical level shows a similar variation. With the critical level in mind, much that was dark becomes clear and reasonable. Above the critical level, blood may be drawn by the hundred cubic centimetres without shock—at the critical level, the loss of 50 cc. may be fatal. Upon the operating table, the loss of a small quantity of blood may sometimes cause shock, whereas a pint to a quart may be taken from a healthy young man without serious injury. The difference lies in the condition of the circulation at the time the blood is lost. If the arterial pressure is near the critical level, small losses of blood upon the operating table become highly important. This is especially true if the bleeding be from an artery. Arterial bleeding is more dangerous than bleeding from a vein; if the arterial pressure is at the critical level, the arteries are partly empty; to empty them still further may be mortal; important structures, such as the respiratory nerve cells, are fed from the arteries rather than from the veins. Finally, the nutritive metabolism of the higher nerve cell units, particularly the respiratory cells, may be so affected that 50 cc. of blood will only do the work of 10. The loss of blood is then a grave disaster. For the critical level varies with the condition of the nerve cells and other tissues. A blood pressure high enough to maintain a sufficient nutrition in normal bulbar nerve cells may be too low to maintain function in cells which have already suffered from malnutrition.

The failure to comprehend the critical level has led many surgeons to believe that hemorrhage is a frequent cause of shock upon the battlefield. This is far from the case. In my field experience, at the Massif de Moronvillers and the Chemin des Dames, shock appeared approximately once in every hundred noteworthy casualties, and then chiefly after fractures of the long bones; among the other ninety-nine there were practically always men who had bled more than the shocked, but who nevertheless had no shock.

It has been stated above that the symptoms of shock appear when the hemorrhage has sufficiently lowered the general blood pressure and that if at this time, the state of the vasomotor cells be tested quantitatively by the stimulation of afferent nerves, the vasomotor cells will be found still normal. Such tests were systematically applied in 1907 and 1908.[19]

[18] The conception of a "critical level" of the blood pressure in shock was first used by me in a communication to the Paris Académie des Sciences, Comptes rendus, t. 163, p. 492, séance du 30 octobre, 1916. See also, Boston Med. and Surg. Journ., 1916, clxxv, 854–858; and Ibid., 1918, clxxviii, 657–660.

[19] This Journal, 1907, xx, 399–405; Ibid., 1908, xxi, 460–465.

They showed a normal reflex change in arterial pressure on stimulating afferent nerves in animals in which the pressure had fallen to shock levels in consequence of hemorrhage. For example, five cats and one rabbit were bled and the branchial or sciatic nerves then stimulated, with the following results.

| BLOOD PRESSURE AT THE BEGINNING OF STIMULATION | NUMBER OF OBSERVATIONS | AVERAGE PERCENTILE RISE IN BLOOD PRESSURE |
|---|---|---|
| *mm.* Hg | | *per cent* |
| 71 to 80 | 4 | 71 |
| 61 to 70 | 6 | 73 |
| 51 to 60 | 5 | 67 |
| 41 to 50 | 10 | 72 |
| 31 to 40 | 6 | 76 |

Similar results were obtained with the depressor nerve.

For many years injury to the abdominal vessels has been used to produce shock. The abdomen is opened and the viscera are roughly handled, or the exposed intestines are blown upon by currents of warm moist air. Whatever the procedure, the result is the same; the largest vascular area in the body is dilated, the general arterial pressure hydrostatically falls, and the classical symptoms of shock appear. But the vasomotor cells are at this time still normal, as shown in the experiments of 1903, already presented.

It must be recognized that low blood pressures do not act upon different tissues in the same way, nor is their action synchronous. The vasomotor cells are not the first to be affected by low blood pressures. A pressure low enough to produce shock may not affect the vasomotor cells except after a period often surprisingly prolonged. Death itself may take place while the vasomotor cells are still alive and uninjured. In the following case, the normal absolute fall of blood pressure upon stimulation of the depressor nerve was secured in a rabbit which had died of diphtheria.[20]

*Experiment January 20, 1914.* A rabbit weighing 1400 grams received in the ear vein 0.004 cc. diphtheria toxin at 1:05 p.m., January 18. The morning of January 20 the rabbit seemed listless. It was placed on a table and observed continuously from 9 a.m. As the day wore on, the rabbit could not hold up his head, nor regain his feet when laid upon one side. Finally, about 3:15 p.m., he lay prone, the head stretched on the table, and the respiration feeble and labored. At 3:30 p.m. he seemed so near death that he was placed on the operating board. Death at once followed; there was no corneal reflex, no respiration, no heart beat, the carotid artery seemed empty, and the rectal temperature was 32°C. The rabbit was completely insensitive. It was quickly tracheotomized and artificial respiration was established. Warm normal saline solution was injected into the external jugular vein. The heart began to beat, though feebly, scarcely raising the writing point of a membrane manometer, com-

[20] Porter, W. T., and J. H. Pratt. This Journal, 1914, xxxiii, 439.

pletely undamped. The carotid pressure rose to about 80 mm. Hg. Both vagi were now cut and the depressor nerve was stimulated. The pressure fell from 80, 70 and 72 mm. Hg to 52, 40 and 45 mm., respectively, an absolute fall of 28, 30 and 27 mm. Hg, and a percentile fall of 35, 43 and 38.

It need hardly be said that in this dead rabbit the pressure was even lower than in living cases of shock from hemorrhage or from dilatation of the abdominal vessels. A general anemia does not necessarily entail a serious anemia of the vasomotor region. The vasomotor cells will function normally on a very limited quantity of blood, and for this quantity they are not entirely dependent on the general arterial pressure.

In dealing thus with hemorrhage and with dilatation of the abdominal vessels as causes of shock without injury of the vasomotor cells, we do not deny that very low blood pressures will in the end cause death and that when the final scene draws near the vasomotor cells may suffer with all the other tissues. If afferent nerves, such as the depressor, are then stimulated, the response of the vasomotor cells will be less than normal. But this research deals primarily with shock and not with the *descensus Averni*, and these pages prove beyond question that in the first order of shock the complete clinical picture is present long before the ante-mortem injury of the vasomotor cells.

*Separation effects.* We pass now to the cases in which important vascular areas are dilated by interrupting the vasomotor paths which connect them to the vasomotor centre. Separation effects were seen by the writer in 1916 in the great hospital at Amiens. Wounds of the spinal cord had severed the vasomotor paths. The thigh and leg on the injured side were warmer and redder than on the uninjured side. The enemy's bullet had done in man that which Claude Bernard, Schiff and Brown-Séquard had done on animals sixty-five years before, when they discovered that section of the vasomotor nerves was followed by a long congestion of the limb thus separated from the vasomotor centre. The wounds just cited, and the operations of Claude Bernard and his contemporaries, caused arterial dilatation in a limb, an area too small to affect the general blood pressure. In the cat, the vasomotor nerves to all four limbs and to the head may be severed without a noteworthy fall in the general blood pressure. This is substantially true in other animals. Only separation injuries of the splanchnic area need concern us here; these alone cause a dangerous fall in blood pressure. Ludwig and Cyon,[21] in the paper which records the discovery of the depressor nerve, observed that section of both splanchnic nerves reduced the blood pressure to 31.5 mm. Hg. The section of these nerves dilates the largest vascular area in the body. The low pressures thus produced soon lead to shock.

[21] Cyon, E. and C. Ludwig. Arbeiten aus der physiologischen Anstalt zu Leipzig vom Jahre 1866, 128–149.

Separation of the abdominal area from the vasomotor centre is seen also when local anesthetics are applied to the connecting paths. The resulting dilatation of the abdominal vessels may cause the symptoms of shock with startling rapidity. I have recorded one such instance, observed in La Panne, Belgium, during my service there in 1916. It was a case in which novocaine was injected into the vertebral canal to secure local anesthesia for the cleansing of a shell wound of the thigh. Novocaine injected into the vertebral canal will sometimes pass high enough to reach the vasomotor paths which supply the abdominal vessels. This man's systolic arterial

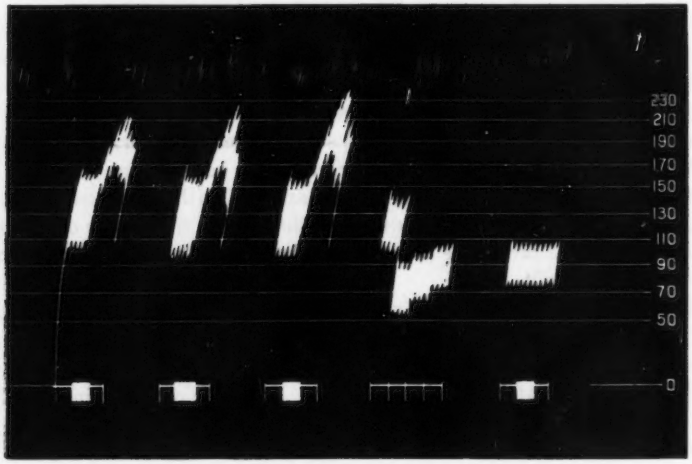


Fig. 10. *February 26, 1915.* The original size. The carotid blood pressure in a curarized cat. The injection of 0.1 gram novocain and adrenalin beneath the dura at Lumbar VII interrupts the nerve paths between the vasomotor centre and the splanchnic area.

pressure was 140; the diastolic, 92 mm. Hg. When the novocaine anesthetized the spinal paths and thus severed the physiological connection between the vasomotor centre and the abdominal vessels, the symptoms of shock at once appeared. The pulse suddenly failed. It could no longer be felt at the wrist. The face and body became deathly pale. The whites of the eyes showed. The man was scarcely conscious. Treatment was by heat, the inclined position, and venous injections of warm normal saline solution with adrenalin. No doubt these measures were useful, but recovery did not take place until from sixty to ninety minutes, time enough to weaken the effect of the local anesthetic.

The mechanism of the fall in blood pressure in this man is shown by the following protocol of an experiment upon a cat.[22]

[22] Smith and Porter. This Journal, 1915, xxxviii, 111.

*Experiment February 26, 1915.* A membrane manometer was connected to a cannula in the carotid artery of a curarized cat. The injection of 0.01 gram novocaine and adrenalin in dilute solution (1 cc.) beneath the dura at the seventh lumbar vertebra paralyzed the dorsal columns as far as Dorsal XI, and perhaps above. The diastolic arterial pressure fell from 110 mm. to 57 mm. Hg. The details are shown in figure 10. The first record (at the left of fig. 10) was a sciatic stimulation at 12:10 p.m. The next was a brachial stimulation at 12:14. The third was a stimulation of the spinal cord at Lumbar VII, 12:15 p.m. Then follow four records at 12:17, 12:25, 12:28 and 12:31 p.m. Between the first and second of these (12:22 p.m.) the novocaine and adrenalin mixture was injected. The pressure fell from 110 mm. to 57 mm. Hg, after which it rose to 74 mm. Hg. The last record (extreme right) was a sciatic stimulation, 12:32 p.m. The reflex has disappeared.

The fall of arterial pressure in this experiment, and in the surgical case cited above, was due to the separation of the vasoconstrictor centre from the abdomen and the hind limbs. The connecting paths were blocked by the novocaine. That the bulbar vasomotor centre in such cases is still normal is proved by experiments made in 1899. The splanchnic nerves were severed in a rabbit, causing the same great fall in blood pressure as in the experiment illustrated by figure 10. The normal arterial pressure was then restored for a time by injections of normal saline solution and both depressor nerves were stimulated. The response was as great as before the splanchnic nerves were cut, showing that the vasomotor centre was unimpaired. An abbreviated protocol[23] follows.

*Experiment October 21, 1899.* In a rabbit anesthetized with a mixture of ether and alcohol a thread was passed around each splanchnic nerve. The carotid blood pressure was recorded by a membrane manometer. The central ends of both depressor nerves were now stimulated simultaneously. The blood pressure fell from 80 mm. to 60 mm. Hg (25 per cent) and returned to 80 mm. when the stimulation was stopped. The splanchnic nerves were now torn through by means of the threads which had been passed around them. The blood pressure thereupon fell to 60 mm. Through a cannula in the right jugular vein 0.8 per cent sodium chloride solution at 38°C. was injected until the blood pressure rose to 85 mm. The depressors were then stimulated again, with the following result:

| BLOOD PRESSURE BEFORE STIMULATION OF DEPRESSOR NERVES | LOWERED BY STIMULATION TO | PERCENTILE FALL |
|---|---|---|
| *mm.* Hg | *mm.* Hg | *per cent* |
| 85 | 65 | 24 |
| 85 | 58 | 32 |
| 88 | 60 | 32 |
| 85 | 58 | 32 |
| 86 | 60 | 30 |
| 85 | 60 | 29 |
| 90 | 60 | 33 |
| 88 | 55 | 37 |
| 90 | 57 | 37 |

[23] Porter and Beyer. This Journal, 1900, iv, 292.

Thus in separation shock also, the vasomotor centre is normal.

In the second order of pathological conditions producing shock the vasomotor cells are from the first directly attacked; by concussion, embolism, or poisons.

*Concussion or vibration injuries.* Vibration injuries are illustrated by an experiment made in 1905, the protocol[24] of which is as follows:

*Experiment March 24, 1905.* The stimulation of the brachial and sciatic nerves in an etherized cat, sufficiently curarized to paralyze the motor nerves, caused an average rise of 27 per cent in the carotid blood pressure. After a heavy blow on the skull, the blood pressure fell from 140 mm. to 35 mm. Hg. At this level the stimulation of the brachial and the sciatic nerves had little effect, the pressure rise being only 3 mm. Hg. After an hour, the carotid pressure rose spontaneously to 90 mm., and then to 130 mm. At these levels, three stimulations caused an average rise of 30 per cent. The autopsy showed fracture in the left frontal region, extending to the base of the skull, and hemorrhage beneath the seat of fracture.

In this experiment the concussion shook the vasomotor cells out of function.

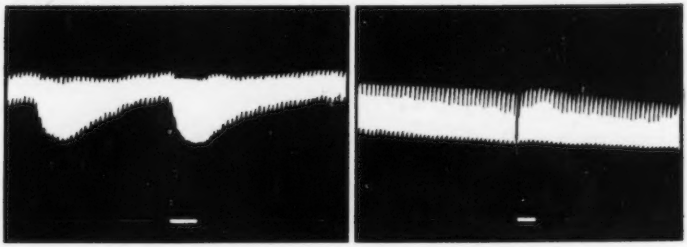

Fig. 11. *December 29, 1923.* Four-fifths the original size. The vasomotor centre does not react to depressor stimulation in fat embolism shock. Carotid blood pressure in a rabbit. At 11:26 a.m. (left curve, second stimulation), the left depressor was stimulated. The diastolic arterial pressure fell from 140 to 95 mm. Hg. At 11:29 a.m., 0.6 cc. neutral olive oil was injected into the left subclavian artery, which had been tied on both sides of the origin of the vertebral. A portion of this oil reached the vasomotor region through the vertebral. At 11:39 (right curve), the depressor was again stimulated; there was no response.

*Embolism of the vasomotor region.* Embolism of the vasomotor region has been sufficiently described in section IV. Next in order are the experiments which demonstrate that in fat embolism shock the vasomotor centre is itself attacked. Such a demonstration is given in figure 11, and in the following protocol.

*Experiment December 29, 1923.* In a rabbit weighing three kilos the carotid blood pressure was recorded by a membrane manometer. At 11:26 a.m. the left depressor nerve was stimulated (see the left hand curve in fig. 11). The diastolic arterial pres-

[24] Porter and Storey. This Journal, 1907, xviii, 186.

sure fell from 140 mm. to 95 mm. Hg. At 11:29 a.m., 0.6 cc. neutral olive oil was injected into the left subclavian artery, between two ligatures tied on each side of the origin of the vertebral artery. Part of the oil remained in the subclavian pouch, part was used to fill the vertebral artery between the pouch and the bulb, and the remainder entered the bulb itself. At 11:39 a.m., the depressor nerve was again stimulated (right half of fig. 11). There was no response. The arterial pressure, which had by this time sunk almost 50 per cent, continued to fall.

The observations illustrated by figure 11 are evidence that in fat embolism shock the vasomotor centre is directly attacked.

Shock due to the action of certain poisons is also to be classed with the types in which the vasomotor centre is itself attacked. The conditions under which these poisons operate, and the character of the symptoms which they call forth, are sufficient to set them in a class apart from early shock in war and in the accidents of peace. We need not discuss them here. Nor need we discuss the complicated and unlimited alterations in tissues and functions which sooner or later inevitably follow profound changes in the distribution of the blood. They are to be regarded as the results rather than the cause of shock.

## IX. TREATMENT

In July, 1916, I went to France for the Rockefeller Institute for Medical Research to study surgical shock in wounded soldiers. Observations were made in *postes de secours*, for example, at Nieuport and the Massif de Moronvillers; and in several field hospitals, including Doctor Carrel's hospital at Compiègne, the hospital of Doctor Du Page at La Panne, the *triage* at Mourmelon-le-petit, and the station at Vauxtin near the Chemin des Dames. At La Panne, my report to Doctor Du Page on the systematic treatment of low blood pressures was issued by him to the staff September 11, 1916 (note no. XXVIII, Sur le traitement du shock chirurgicale). To the procedures therein described was added, in 1917, the use of carbon dioxide to raise the blood pressure by the increased action of the respira tory pump.

The essential points in the writer's systematic treatment of shock are as follows:

From a practical standpoint, shock exists when the diastolic arterial pressure is 60 mm. or less. The remedies are:

1. A special position of the wounded; the abdominal vessels should be higher then the heart and brain.
2. Heat.
3. Intravenous injections of normal saline solution.
4. Intravenous injections of adrenalin.
5. The inhalation of carbon dioxide.
6. The transfusion of blood, in certain cases.

*1. Position.* The patient should be taken directly from the ambulance to a special bed in a room or tent devoted to the treatment of shock. He is on no account to be moved from this bed—moving shock cases may kill them—nor should the specialist detailed for shock cases leave his patient for more than a few moments. Meals should be eaten at the bedside.

The special bed may be of the following description: *a*, The central part is of a size identical with a surgical operating table. *b*, The removable side pieces increase the width to that of the ordinary hospital bed. *c*, The foot of the bed must be raised 30 cm. The pillow must not be more than 6 cm. high. *d*, The bed is heated electrically from below. *e*, When

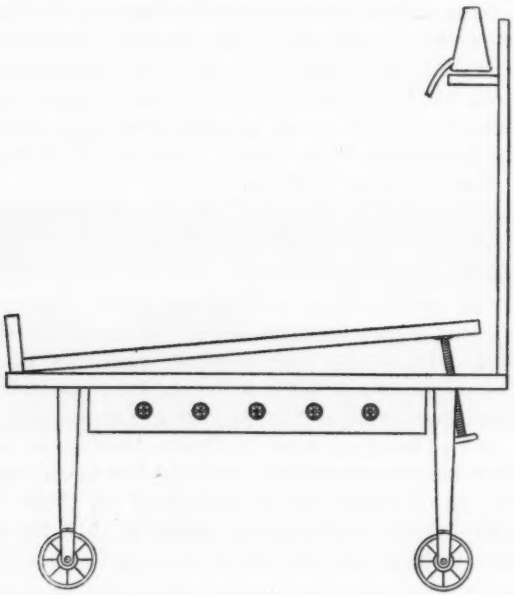

Fig. 12. A combined bed and operating table for shock patients.

the side pieces are lifted off, the bed is transformed into an operating table. *f*, A stand 1.5 metres above the patient supports a bottle of normal saline solution, kept at 39°C. by an electric heater (fig. 12).

*2. Heat.* The heat should be continued until the feet and hands of the patient are warm. The heat should then be turned off, at least for a time.

*3. Normal saline solution.* *a*, When the diastolic pressure is below 80 mm., normal saline solution should be injected into a vein. The fluid must be at 38°C. when it enters the vein. About 500 cc. may be injected, but it is not desirable to continue the injection after the diastolic pressure has

reached 80 mm. The injection should be made slowly, lasting about 10 minutes. *b*, If the pressure falls some time after the first injection is given, a second injection of normal saline should be made. The quantity should again be 500 cc., provided a diastolic pressure of 80 mm. is not reached with less, and the injection should occupy about 10 minutes.

*4. Adrenalin.* If the pressure falls below 80 mm. after the second injection of normal saline solution, adrenalin should be injected into another vein. The adrenalin, in a solution of 1:1000, should be kept in a stoppered flask in the dark. Solutions not colorless should be rejected. At the moment of injection, mix 0.5 cc. of the adrenalin solution in 50 cc. of normal saline solution, at 38°. The injection should be made very slowly, and should be suspended if the heart becomes irregular.

*5. Carbon dioxide.* If the diastolic pressure cannot be kept above 80 mm. with normal saline and adrenalin, carbon dioxide should be added to the inspired air until the frequency of respiration is doubled. The oxygen content of the inspired air should remain at normal.

*6. The transfusion of blood.* Occasionally there are cases too low for immediate operation and in whom death is threatened from slight but persistent hemorrhage. Normal saline injections or adrenalin would increase the hemorrhage by increasing the blood pressure. Such cases demand the transfusion of blood. Some of the blood transfused will be lost by the hemorrhage, but enough may be retained to bring the patient to the point at which the vessels may be tied and the usual treatment for shock instituted.

The success of this treatment, and in skilled hands it is markedly successful, rests upon new principles: *a*, the perception that the diastolic rather than the systolic arterial pressure should be the basis of diagnosis and treatment;[25] *b*, the recognition of a critical level of the diastolic blood pressure in shock;[26] *c*, the maintenance of the diastolic blood pressure somewhat but not too much above the critical level as determined by very frequent readings of the diastolic arterial pressure.[27] These new principles we must now briefly discuss.

*a. The diastolic vs. the systolic pressure.* Practitioners who are not special students of the circulation may easily overlook certain fundamental truths in the relation of diastolic to systolic pressure. Measurements made July 22, 1892,[28] in a dog's heart beating 92 to the minute, gave for the duration of systole 0.290 second, while the duration of diastole was 0.363 second. The cardiac cycle, or interval from the beginning of one ventricular contraction to the beginning of the next, was therefore 0.653 second.

[25] Boston Med. and Surg. Journ., 1918, clxxviii, 657–660.
[26] See note to p. 302.
[27] Boston Med. and Surg. Journ., 1918, clxxviii, 657–660.
[28] The (English) Journ. Physiol., 1892, xiii, plate xix, fig. 25b.

The aortic valves were open during approximately two-thirds of systole, or 0.190 second, which is 29 per cent of the cardiac cycle. In other words, the ventricle was open 29 per cent of the time and closed 71 per cent of the time. In the twenty-four hour day, the ventricle (in a heart beating 92 per minute) was closed 17 hours and was open only 7 hours. Thus the circulation, in this frequent heart, was carried on solely by the diastolic pressure seventeen hours out of the twenty-four.

Moreover, the systolic pressure is measured clinically in the large arteries, but the nerve cells, upon whose nutrition success or failure hangs in early shock, are fed from the capillaries and not from the large arteries. The systolic pressure is lost in the capillaries.

In short, the systolic pressure is of value in keeping a high diastolic pressure in the large arteries—a central rather than a peripheral function. It is the diastolic pressure which moves the blood and feeds the cells.

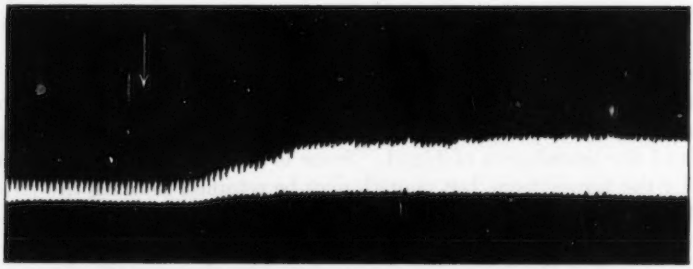

Fig. 13. *December 29, 1923.* The original size. Showing the gross error which might follow the use of the systolic arterial pressure to measure the effect of normal saline injections. Carotid pressure in a rabbit. Oil had been injected into the vertebral artery, and the diastolic pressure had fallen from 135 to 35 mm. Hg. At the arrow (fig. 12), 70 cc. warm normal saline solution were injected into the jugular vein. The diastolic pressure was thereby raised 4 mm. Hg, but the systolic pressure was raised 50 mm. Hg, i.e., from 45 mm. to 95 mm. Hg.

Hence, it is with the diastolic rather than the systolic arterial pressure that we should deal in the treatment of shock. Finally, gross errors are made by using the systolic pressure as a guide to the value of remedial measures. Figure 13 is evidence of this. It is from the experiment of December 29, 1923.

*Experiment, December 29, 1923.* The carotid pressure of a rabbit was written by a membrane manometer. Oil was injected into the vertebral artery. The diastolic arterial pressure fell from 135 mm. to 35 mm. Hg. Figure 13 begins at 35 mm. pressure. At the arrow (fig. 13), 70 cc. warm normal saline solution were injected into the jugular vein. The diastolic pressure was thereby raised 4 mm. Hg, but the systolic pressure was raised 50 mm. Hg, i.e., from 45 mm. to 95 mm. Hg.

For all these reasons, the diastolic and not the systolic pressure should be used to determine the critical level in shock.

*b. The critical level.* The critical level in shock was discussed in section VIII, pages 301 and 302.

*c. Raising the diastolic pressure.* The diastolic pressure may fall from normal to the critical level with little danger—a further fall of even 10 mm. may be fatal, unless skilled assistance be at hand. Conversely, in dangerous shock, lifting the diastolic pressure 15 mm. will save life, as a rule. Measures which will raise the diastolic arterial pressure 15 mm. are therefore usually adequate. Increased action of the respiratory pump, advocated in 1917, is such a measure.[29]

When the diaphragm descends in inspiration, the cavity of the thorax is enlarged. It is as if a squeezed rubber bulb were expanded under water; the surrounding fluid enters the sucking ball. So do surrounding fluids enter the chest. The air is sucked in through the trachea and blood is sucked in through the veins. In man, this suction may balance a column of mercury 30 mm. high, equal to a column of blood 15 inches high—a value one-third the total normal diastolic arterial pressure.

If the normal contractions of the diaphragm so aid the circulation, its powerful contractions will aid still more. Powerful and frequent contractions are within our command. We have but to increase the carbon dioxide in the inspired air to call forth deep and rapid respiration. The necessary amount of carbon dioxide is not injurious.

I have raised the diastolic blood pressure with carbon dioxide in normal men, in animals with experimental fat embolism, and in soldiers with shock.

Anyone can easily raise the diastolic blood pressure 10 to 15 mm. Hg in a normal individual by increasing the carbon dioxide in the inspired air until the respiration is doubled. The necessary amount of carbon dioxide is about 3 per cent; it should be mixed with sufficient air to prevent oxygen hunger.

The observations on animals are illustrated by two experiments April 14, 1917. The carotid pressure in cats A and B was written by a mercury manometer. In these cats 1 cc. of olive oil per kilo of body weight had been injected into the external jugular vein. In consequence of the fat embolism thus produced, the blood pressure was falling. On increasing the carbon dioxide in the respiratory air, the diastolic pressure rose 20 to 30 mm. Hg.

The following cases[30] of shock observed at Vauxtin illustrate the respiratory treatment in man:

[29] First proposed in the Boston Med. and Surg. Journ., 1917, clxxvi, 699.

[30] Compt. rend. Acad. d. Sci., Paris, clxv, 164, séance du 23 juillet, 1917; also, Boston Med. and Surg. Journ., 1917, clxvii, 326–328.

Case 1. June 25, 7 a.m. Both legs amputated. Diastolic arterial pressure 51 mm. When carbon dioxide was inhaled until the quantity of air entering the chest was about doubled, the diastolic pressure rose to 60 mm. At 11 a.m. the patient was out of danger.

Case 2. June 26, 8:25 a.m. Two deep wounds in the back. Multiple wounds elsewhere. Diastolic pressure, 53 mm. Inclined position and hot normal saline in vein caused pressure to rise to 70 mm. Operation at 10:15 a.m., lasting a quarter hour. At 10:30 the diastolic pressure was 52 mm. An injection of adrenalin brought it to 57 mm. for a short time only. At 11:05 the pressure was 53 mm. At 11:15 the respiration was deepened by inhaling carbon dioxide; 11:20, diastolic pressure 60 mm.; 11:25, carbon dioxide was stopped and the pressure thereupon fell to 53 mm. At 11:35 the gas was again employed and the pressure rose to 61 mm. This man recovered.

Case 3. June 29, 6 a.m. Right leg crushed. Many small wounds through subcutaneous fat. Diastolic pressure 47 mm. Injections of normal saline solution in vein at elbow did not raise the pressure. Ether injected under the skin also produced no effect. Increased respiration from inhaling carbon dioxide at once increased the pressure. The pulse, which could scarcely be felt at the wrist, became plainly stronger. At about 11:30 a.m., during carbon dioxide breathing, the leg was amputated under local anesthesia, and the multiple wounds dressed without anesthesia. There was no unfavorable reaction, though under ordinary conditions (without carbon dioxide respiration) the operation would almost certainly have been fatal. Several hours after the operation, this man's femoral pulse and heart action were so good that he was believed out of danger. The carbon dioxide respiration was discontinued. In about ten minutes, the respiration became feeble and the pulse less strong. The carbon dioxide was at once renewed, but the respirations did not become stronger, and in ten minutes more the man was dead, in spite of the carbon dioxide atmosphere. He was from the beginning a case ordinarily called hopeless.

These examples of my observations on normal men, on animals in whom the blood pressure has begun to fall from fat embolism, and in wounded men with severe shock, are evidence that the inhalation of carbon dioxide, in accordance with physiological rules and controlled by repeated measurements of the diastolic arterial pressure, is a useful addition to the remedies for early traumatic shock. Carbon dioxide will often raise the diastolic pressure from the danger level to the safety level.[31]

## SUMMARY

1. The introduction describes early traumatic shock, and states the purpose of this investigation (section I).

2. Early shock, seen typically in freshly wounded soldiers, is most frequent after shell wounds of the long bones (section II).

[31] In addition to the papers cited in the text the writer has published articles on shock in the Boston Medical and Surgical Journal, 1917, clxxvi, 248; 1918, clxxix, 273–274; 1919, clxxx, 531–532; and in the Proceedings of the Institute of Medicine in Chicago, 1918, ii, 24–29.

3. Injuries of the long bones cause fat embolism. Clinical evidence does not prove that this fat embolism is a cause of shock, since possible causes other than fat embolism are present in every clinical case of shock. Experiments on animals are necessary (section III).

4. Experiments are given in which shock is produced by fat embolism alone. Other experiments are presented in which shock is produced by fat embolism limited to the vasomotor region (section IV).

5. If embolism of the vasomotor region is a cause of shock, the injection of oil into the carotid artery might *a priori* be expected to cause shock. It often does not. The reasons are given in section V.

6. In clinical cases of fat embolism of the bulb, fat embolism of the lungs is always present. But pulmonary fat embolism is not a cause of shock (section VI).

7. Shock is not due to exhaustion of the vasomotor centre, in the sense of fatigue from over-stimulation. Nor is shock due to failure of the vasomotor centre, except when caused by bulbar fat embolism, by concussion of the bulb, or by certain poisons. In shock of other types, e.g., from abdominal injuries, the vasomotor cells are normal (section VII).

8. Shock is the final common scene in various and quite distinct pathological states. In the first order of these, the symptom complex is produced without injury to the vasomotor centre. In this first order are hemorrhage, dilatation of the abdominal vessels without injury to the vasomotor paths, and injuries which sever the vasomotor paths running to the splanchnic area. In the second group of pathological states leading to shock, the vasomotor centre is from the beginning directly attacked. This group includes concussion of the vasomotor cells, fat embolism of the vasomotor region, and the action of certain poisons on the vasomotor cells. These several types of shock are contrasted in section VIII.

9. A systematic treatment of shock, used with marked success on wounded soldiers, is described in section IX. This treatment is grounded on new principles: *a*, the perception that the diastolic rather than the systolic arterial pressure should be the basis of diagnosis and treatment; *b*, the recognition of a critical level of the diastolic blood pressure in shock; *c*, the maintenance of the diastolic blood pressure somewhat but not too much above the critical level, as determined by very frequent readings of the diastolic pressure. Measures which raise the diastolic pressure in shock even 15 mm. may save life. A new remedy, often successful in raising the diastolic pressure to this extent, is found in the increased action of the respiratory pump, secured by augmenting the proportion of carbon dioxide in the inspired air.

# STUDIES ON THE RELATION OF THE EXTERNAL TO THE INTERNAL SECRETION OF THE PANCREAS

## II. The Effect of Trypsin on Insulin and its Bearing on the Causation of Diabetes

ALBERT A. EPSTEIN and NATHAN ROSENTHAL
with the collaboration of
EUGENIA H. MAECHLING and VIOLET de BECK

*From the Laboratory of Physiological Chemistry, Pathological Department, Mount Sinai Hospital, New York City*

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Diabetes mellitus is believed to be due to a deficiency of the internal secretion (insulin) of the pancreas, resulting from a functional or structural change in the islands of Langerhans. In clinical and experimental evidence, however, we find many contradictions to this conception of the pathogenesis of the disease. Better proof is needed than that available for the assertion that structural changes in the pancreas lead to functional incapacity, and that functional impairment is capable of producing changes in structure.

Pathological changes in the islet cells in certain cases of human diabetes have been described by a number of observers (1), (2), (3), (4), (5), (6), but there is no unanimity of opinion regarding the existing material. While it may perhaps be said that advanced cases of diabetes frequently show certain changes in the pancreas, no such changes are encountered in the milder forms of the disease. Allen (7) observed that the production of hyaline degeneration in the B cells of the islets was coincident with a breakdown of carbohydrate tolerance.

Influenced by these observations, the belief has gained ground that the structural changes noted in the islands of Langerhans, in advanced cases of diabetes, represent the end result of a functional strain. But it should be remembered that the changes referred to are of a degenerative type, and hence the possibility must be borne in mind that they may be due to some cause other than the mere effort to produce insulin.

Hyaline degeneration of the islet cells, very similar to that occurring in diabetes, has been observed in non-diabetics, especially in older persons and cachectics (8). Joslin (9), quoting Mallory, states that: "In various acute infectious processes, such as lobar pneumonia and diphtheria, mitosis and regeneration of the cells of the islands are of common occurrence."

In experimental work on rabbits, Thomas (10) found that general infections by certain bacteria produce changes in the pancreas, particularly in the islands of Langerhans.

In other words, pathological changes in the pancreas resembling those of diabetes can occur in human subjects and in experimental animals, which are neither due to a lack of insulin nor to any strained effort to produce it; furthermore, such changes in themselves are evidently not capable of producing diabetic manifestations.

In the introductory remarks to chapter X of his book, Allen (11) makes the following significant statement: "But it is striking what advanced degeneration of the pancreas may thus be produced, without diabetes, in contrast to the relatively slight changes often found in diabetic men and animals in connection with spontaneous diabetes."

It is conceivable, therefore, that the changes referred to are merely incidental and constitute the result (directly or indirectly) of those injurious or toxic agencies which either cause or follow the diabetes. Be that as it may, the interesting fact remains that in the moderate or mild cases of diabetes, the histology of the pancreas fails to show any deviation from the normal.

That insulin is the active principle of the internal secretion of the pancreas, and that it is directly concerned with the metabolism of carbohydrates, can no longer be doubted. It is certain, too, that diabetes represents a disturbance of carbohydrate metabolism directly traceable to a deficiency of the internal secretion of the pancreas. The question follows: What is the cause of this deficiency?

Complete removal of the pancreas in an animal produces a total diabetes and, to produce a condition resembling clinical diabetes, even of mild degree, nearly all of the pancreas must be removed. In experimental animals mere shreds of pancreatic tissue are said to be sufficient to hinder the incidence of the disease. This indicates, in the animal at least, that only a small portion of normal pancreatic tissue is necessary to supply insulin adequate to the needs of normal carbohydrate metabolism.

Intolerance of carbohydrate, hyperglycemia and glycosuria constitute diabetes, admittedly due to a lack of insulin; but does that signify that every case of human diabetes represents a crippling of the major part of the functional activity of the islands of Langerhans?

Experience teaches that only small doses of insulin are needed to control glycosuria in mild cases of diabetes, whereas in severe cases the requirement may be very great. The degree of deficiency as represented by the two types of diabetes mentioned above is vastly different, yet we must assume on the basis of experimental evidence that the actual functional impairment in the severe type is but slightly greater than it is in the mild one.

It is obvious, therefore, that the existing conception of the etiology of the disease is inadequate and that another interpretation of the facts is needed. The causation of human diabetes cannot be satisfactorily explained by the knowledge gleaned from total or subtotal extirpation of the pancreas in animals. This knowledge merely serves to affirm that the pancreas is concerned with carbohydrate metabolism and that the removal of all or the greater part of it creates a deficiency of the substance necessary for normal utilization of sugar.

In the human subject the amount of reduction of the functionating pancreatic tissue which is capable of causing diabetes has never been determined. Diabetes rarely follows pancreatitis. Cysts and tumors of the pancreas do not cause diabetes although they frequently involve and destroy large portions of the gland. In discussing the surgical aspects of pancreatic neoplasms, Gilbride (12) makes the following remarks: "It is not definitely known just how much pancreatic tissue must be left in the body in order to provide for the internal secretion of the gland. If we are to judge by our clinical experience, it is not necessary to leave a large part of the gland, because glycosuria is only rarely present in cases of tumor of the pancreas. I have observed an absence of glycosuria even when the entire pancreas appears to the naked eye to be cancerous. This suggests that either the islands of Langerhans escape cancerous invasion, or the few remaining islands seem to be capable of caring for carbohydrate metabolism."

Best, Smith and Scott (13) have recently shown that insulin is almost universally present in the tissues of the body, and that the amount of insulin secured from the tissues of depancreatized animals, long after removal of the pancreas, is greater than that present in the pancreas itself at any one time. They assume that this widely distributed insulin exists in the tissues in an "inactive" form and for this reason fails to exhibit its physiological action.

The facts, therefore, do not warrant the supposition that diminished production of insulin is the sole cause of insulin deficiency. Such a "deficiency" may have at least two sources: it may arise from an actual decrease in the production of insulin, or it may be due to a neutralization or destruction of insulin.

If inactivation of insulin can take place in the different tissues of the body, it can also occur in the pancreas, particularly as the products of its external secretion are of such a character that their contact with insulin would favor the neutralization of insulin within the gland itself.

In a previous communication (14) we have shown that trypsin inactivates insulin, and that it can accomplish this within the body of an animal as well as in the test tube. We have been led by these considerations to the conclusion that trypsin, which is present in the external secretion of

the pancreas, may play a part in the causation of diabetes. This is contrary to the views generally held. Current opinion insists that the external secretion of the pancreas bears no relation to the causation of diabetes. Joslin (15) states categorically that: "The external secretion of the pancreas exerts no influence upon the etiology of diabetes." This viewpoint we find is merely based on opinion and belief which lacks confirmatory evidence. On the contrary, there are many indications that the external secretion of the pancreas may, under certain conditions, influence the internal one.

Genetically the structures forming the islands of Langerhans are closely related to the acinar glands. Islet cells are known to develop from the walls of the pancreatic ducts. Some investigators are of the opinion that islet cells may develop from the cells of the acini. In a recent review of this question Vincent (16) arrives at the conclusion that the islands of Langerhans are not separate and distinct organs. According to this author, the islands represent temporarily modified portions of the secretory tubules of the pancreas, and the tinctorial differences between the cells merely point to different stages of cell activity and not to separate types of cells. Whereas definite proof of this is lacking, it seems certain that the islet and acinar cells have a common origin, namely, the duct epithelium (17), (18). Destruction of the acinar structures, as by plugging or ligation of the ducts, favors the proliferation and development of islet tissue. Exhaustion of the zymogenic cells by means of secretin (19) or prolonged starvation (20) is also said to favor the development of the islet cells, and by inference, their secretion. In other words, various influences or processes which control or eliminate the activity of the acinar cells promote the regeneration and activity of the islet tissue. In a conversation with Colonel Bailey K. Ashford, he stated that: "In sprue (a disease associated with exhaustion of the external secretion of the pancreas) diabetes is of uncommon occurrence and that the blood sugar content in this disease tends to be rather low." This suggests the possibility that under certain conditions the reverse may also take place, that is to say, that overactivity of the pancreatic acini or the passage of their products inward toward the islet structures, or into the blood stream, may affect the generation or the activity of the internal secretion. For example, procedures which promote the free flow of pancreatic juice into the duodenum, (intraduodenal administration of ether) cause a reduction in the blood sugar which is interpreted as signifying that the internal secretion is simultaneously increased (21); while inhibition of the flow by such agencies as thyroid, hypophyseal extract and adrenalin produces the opposite effect (22).

Histologically, there appears to be nothing to prevent the passage of the external secretion inward, excepting the fact that outward flow offers less resistance. It is contended that the islands of Langerhans are in

anatomical continuity with the surrounding acinar cells (23). The barrier between the islands of Langerhans and the acini, in the higher mammals, appears to be constituted principally of the endothelial walls of blood and lymph capillaries. The proximity of the islands of Langerhans to the pancreatic ducts is also dangerously close.

The effete products of metabolism of the acinar cells must be carried off by the same capillaries that drain the islands of all their products, including insulin. Alteration in the permeability of the walls of blood capillaries is known to occur. Such changes can affect the character and composition of fluids which leave and enter the blood stream. The unity and close relationship of the vascular supply to both the acinar and islet structures of the pancreas is such that changes in the permeability of the capillary walls could conceivably permit the passage of insulin into the areas occupied by the acinar cells and of trypsin and other constituents of the external secretion inward into the blood. In the first instance[1] insulin would be present in the external secretion, while in the latter the products of external secretion would participate in the internal secretion of the pancreas.

Certain evidence can be adduced in support of this conception. For example, in perfusion experiments of the pancreas, which Clark performed (24), he failed to find any appreciable amount of active insulin in the perfusate. In similar experiments, Murlin and co-workers (25), (26) found that perfusates of the pancreas obtained by means of alkaline solutions exhibited little or no insulin activity, whereas perfusates of acid solutions showed an insulin-like action. Murlin explains his own results and thereby Clark's, by assuming that trypsin is present in all the perfusates; that in the alkaline perfusate trypsin digests insulin, whereas in the acid perfusate, trypsin is prevented from destroying insulin. While as we have recently shown, trypsin does not actually "destroy" insulin, nor does it "digest" it, Murlin's viewpoint is nevertheless correct in principle. We have found that the inactivation of insulin by trypsin takes place at a certain pH. The inactivation is in the nature of a chemical combination which is favored by an alkaline and is hindered by an acid reaction. So that, in an alkaline perfusion, trypsin if present could "inactivate" insulin, whereas in an acid perfusion it would not be able to do so (27).

These perfusion experiments do not prove, of course, that the pancreatic acini secrete trypsin internally, i.e., into the blood stream, but they do indicate two things: 1, that the products of external secretion can pass into the pancreatic circulation (by diffusion or filtration) (28); and 2, that the passage of these products into the blood stream or perfusing fluid (29) causes inactivation of insulin.

[1] This question is at present under investigation.

In pursuing our problem, namely, whether the external secretion and more particularly trypsin, can enter the blood stream, and what the possible effect of such an occurrence would be, we determined first to study the effect of infusion of trypsin directly into the pancreatic circulation in the living animal, without disturbing the pancreas in any way. If in this manner the supply of the internal secretion could be affected, either by injury to the island cells, or by neutralization of the active principle which they produce, we could reasonably expect a disturbance in the carbohydrate metabolism which would manifest itself by a hyperglycemia and glycosuria.

We therefore injected small amounts of trypsin (purified) into arteries supplying the pancreas and found that a hyperglycemia and glycosuria developed promptly (protocol 1). The effect of this and other procedures on the histology of the pancreas will be reported in a separate paper. We are concerned at present only with the functional reactions. Owing to the multiple arterial supply of the pancreas the infusions which we made reached only a portion of the gland and at no time the entire organ. The vessel most commonly used for infusion was the pancreatico-duodenal artery. It is noteworthy that this artery supplies both the pancreas and the duodenum and hence the course of any fluid infused into it is divided in its distribution: some passing into the pancreas, and some into the wall of the duodenum and its mesentery. The ultimate distribution of the infusion fluid is of the utmost importance in the interpretation of our experimental findings; because the result from such a procedure must necessarily be comprised of at least two effects, namely, that arising from the passage of the fluid through the pancreatic branch of the artery and that due to the passage of fluid through the duodenal branch. We found from the infusion of colored solutions (azine dyes) that the distribution of the pancreatico-duodenal artery is very variable, particularly in relation to its duodenal branch, but this matter will be discussed in detail later on. The amount of fluid injected rarely exceeded 2 cc. and frequently was less than that. The rate of injection was slow, and the fluid appeared to be mixed with blood coursing through the gland.

Operations were performed on 85 cats and 2 dogs. Our experiments were divided into three groups. In the first, the pancreatic artery was infused for purposes of histological study, and sections of the affected and unaffected portions of the pancreas were removed at different intervals of time for microscopical examination. In another group the pancreatic artery was infused with different solutions for the purpose of observing the effect on the blood and urine. For reasons which will appear in the text, in the third group of animals infusions were made into the portal vein with each of the substances employed for pancreatic infusion.

Our earlier experiments were performed under ether anesthesia. Because of the fact that ether produces a hyperglycemia, we have in the later experiments used iso-amyl-ethyl-barbituric acid (Amytal, Lilly) to produce anesthesia. As

Page (30) has pointed out, this drug has many advantages over ether in experimental work of this kind, chief of which is the fact that it does not cause any rise in blood sugar.

Technic of operation: As mentioned above, ether anesthesia was used in some of the first experiments. The open mask method and usual procedure were employed. Later when iso-amyl-ethyl-barbituric acid was used, a 2 per cent solution of the drug was injected intraperitoneally, allowing 3.0 to 3.5 cc. per kilogram body weight. Surgical anesthesia was produced in less than 10 minutes and lasted for 18 to 24 hours. Median abdominal incision 2 to 3 inches in length was made from the xyphoid process down. In most instances the pancreatico-duodenal artery was employed for infusion. The pancreas and duodenum were delivered in the wound and the artery exposed to view. By means of dissection with a blunt instrument about half an inch of the artery was liberated from the surrounding structures. The injection was then made with a needle of fine gauge. There was very little manipulation and in most cases the bleeding which followed the removal of the needle was readily controlled. The wound was then closed in two or three layers. This operation in the cat was by no means a simple one, but with a little experience we developed a satisfactory technic and the number of unsuccessful operations was relatively small.

The first effect of injection of trypsin is slight pallor of the affected portion of the gland which is soon followed by a hyperemia. In some instances in which the gland was kept exposed to view for 15 to 30 minutes, a moderate edema of the affected part could be observed.

In view of the brief duration of the infusion and the fact that only a small portion of the pancreas was involved, the incidence and duration of the glycosuria was surprising. Control experiments showed that simple traumatization of the pancreas does not cause a glycosuria; puncture of a pancreatic artery and penetration of the needle into its lumen also do not produce glycosuria.

Several effects might be expected from the infusion of trypsin into the pancreatic arteries: 1, injury to the islands of Langerhans in the affected area with immediate cessation of their activity; 2, direct neutralization of the insulin stored in the islet cells. The brief duration of the perfusion would seem scarcely sufficient to cause any lasting effect; 3, damage to the capillaries, so that some of the external secretion might pass inward after the original trypsin has passed into the general circulation; 4, damage to the liver with glycogenolysis and sugar mobilization.

In order to explain the functional disturbance which was responsible for the glycosuria, we devised a variety of experiments, the results of which supply much of the desired information.

Injections of trypsin into the pancreas via one of its arteries invariably resulted in hyperglycemia and glycosuria and the question arose as to how this was produced. Was this result due to a specific action of trypsin on insulin, or on the insulin-bearing cells? In order to settle the question of specificity in regard to trypsin we next injected normal saline solution into

the pancreatic artery and found that it yielded precisely the same result as trypsin, although the effect was not as enduring as in some of our trypsin-injected animals (see protocol 2). The quantity of saline injected was small, 2 cc., similar to that of trypsin. Simple traumatization could not account for the effect, nor did it seem likely that the saline could do such violent damage to the islet cells or to the pancreas as a whole. Even if this were so, could the injection of a small amount of normal saline mixed with the circulating blood, into a restricted area of the pancreas, so alter the total secretory functions of the island glands as to cause a deficiency in insulin with a consequent hyperglycemia and glycosuria? This did not seem at all reasonable. In the case of trypsin, because of its neutralizing effect on insulin, we could conceive of a temporary deficiency created by its passage into the pancreatic circulation; but the brief passage of a small amount of saline could not conceivably produce any such result. The effect of injecting saline into the pancreatico-duodenal artery was well defined, but the nature of its action seemed obscure. One explanation suggested itself, namely, that by the passage of saline through the pancreatic capillaries, the walls of these capillaries were rendered permeable, so that it became possible for trypsin to flow inward into the blood stream, or for insulin to flow outward toward the acini, thus producing the same deleterious effect on the insulin, but to a somewhat lesser degree than that produced by the injection of pure trypsin.

Current views concerning the function of the internal secretion of the pancreas are so definite that any hyperglycemia or glycosuria resulting from manipulation of the pancreas or any other procedures affecting the pancreas, are immediately attributed to injury of the islands of Langerhans and a diminished secretion of insulin. It seemed difficult to believe that the injection of so small an amount of saline into the pancreatic artery could be responsible for so profound an effect. Even the explanation offered, that the saline in its passage through the blood capillaries permits trypsin to flow inward causing neutralization of the insulin and damage to the islands seems inadequate.

Insulin injected anywhere in the body, subcutaneously or intravenously, in sufficient amounts invariably lowers the blood sugar and controls the glycosuria due to any cause. If the glycosuria and hyperglycemia caused by injection of trypsin solution or saline into the pancreatic artery is the result of a temporary deficiency of the internal secretion, then surely the infusion of insulin into the pancreatic artery should not result either in a hyperglycemia or glycosuria.

To test this, we injected relatively large amounts (20 to 30 units) of insulin into the pancreatic artery of animals and found the effects exactly the same as those produced by trypsin and saline (protocol 3). Insulin so introduced seemed to be without any of its characteristic effects. In

order to eliminate any possible by-effects from the acidity of the insulin[2] it was neutralized or alkalinized in some of our experiments prior to injection, with the identical result, namely, a hyperglycemia and glycosuria.

Any substance introduced into the pancreatic artery must after passage through the pancreas ultimately reach the portal circulation. Is it not possible then that the hyperglycemia and glycosuria which result from infusing substances into the pancreatic artery, really are due to some effect on the liver which causes a glycogenolysis and sugar mobilization?

Viewing the matter from every possible angle, the supposition gained more and more weight, that the glycosuria which resulted from the injection into the pancreatic circulation of trypsin, saline or even insulin, was not directly due to this, but to the incidental mobilization of trypsin into the portal blood stream, with damage to its glycogenetic function.

To test this hypothesis we injected these substances in similar amounts directly into the portal vein and observed the effect produced. We found that whereas trypsin produced a glycosuria, insulin and saline did not (see protocols 4, 5 and 6). Wherefore this difference?

If we correlate these results with those obtained from pancreatic infusion, it would seem that either trypsin or some such substance is carried into the portal circulation by whatever fluid is injected into the pancreatic artery. That the substance which is thus washed into the portal circulation is actually trypsin seems likely from the fact that when insulin is injected no insulin effects are produced, a circumstance which could only arise if the insulin were inactivated or neutralized while passing through the pancreas.

It is worth recalling in this connection the perfusion experiments of Clark (31) and Murlin (32) in which practically no insulin could be found in the perfusate unless, as Murlin ascertained, the perfusion fluid was sufficiently acid to hinder the trypsin from acting on the insulin.

Insulin introduced into the body by any route whatever save that of the pancreatic vessels produces its characteristic physiological effects. It is a most surprising fact, therefore, that by passing through the pancreas, its natural source, it should not only be deprived of its normal powers, but should actually lead to the production of hyperglycemia and glycosuria.

From the fact that insulin introduced into the liver via the portal vein does not cause a glycosuria, and that it does so when passed first through the pancreas, we must conclude that it is not the insulin in the latter instance which is responsible but the trypsin which it carries along with it and which upon reaching the liver causes glycogenolysis and glycosuria.

It would seem therefore fairly certain that under the conditions described, at least, trypsin can pass inward into the blood stream, combine

[2] It will be recalled, however, that acid perfusion of the pancreas (Murlin) favors the recovery of insulin.

with the insulin that may be at hand, and by passing inward into the liver can mobilize sugar.

At this point a number of important questions arise. In the first place, is native insulin secreted in pure active form, or is it associated with trypsin or some such neutralizing agent from which it becomes dissociated through the intervention of another substance? This conception deserves due consideration, as there are a number of factors which point in that direction.[3] Under these conditions the controlling factor in the regulation of insulin activity would not be the islands of Langerhans but the passage of trypsin or some kindred substance into the blood stream along with insulin. In the experiments previously referred to in which insulin was infused into the pancreatic artery, the amount of insulin used was far in excess of that which the pancreas of a cat could possibly produce at any one time, yet the insulin lost all its potency by the passage. It is interesting in this connection to consider the fact that insulin deficiency is a very common condition, whereas hyperinsulinism with its natural consequences is not frequently encountered. It is only recently that such a condition has been described (33).

The second question which arises is: Can any substance pass through the pancreatic vessels without causing a mobilization of the external secretion inward, and hence without the production of glycosuria?

In discussing the nature of the action of trypsin on insulin (34), we suggested that a striking similarity exists between the reaction of trypsin with insulin, to that between trypsin and safranin. From the various points of similarity in the conduct of insulin and safranin in respect to trypsin, we were inclined to believe that the two substances, insulin and safranin, must have some structural unit in common.

Now, if insulin is perfused through the pancreas, it becomes neutralized, presumably becoming attached to trypsin. The question arose as to what would happen if, in place of insulin, safranin were infused into the pancreatic artery.

Before proceeding with our experiments in which perfusion of the pancreas by means of safranin was attempted, it is in place to say a few words concerning the chemistry of safranin and the nature of its action on trypsin. From Marston's admirable work on the subject (35), we learn that safranin combines with trypsin by virtue of its azine and azonium radicals which possess this affinity. He also calls attention to the fact that not only safranin but other dyes possessing an azine and azonium base exhibit the same affinity. The most noteworthy of these is janus green. As the azine and azonium bases are colored substances, the occurrence of the proteolytic enzymes in the cell may be demonstrated by means of their selective staining reactions. This fact has been utilized in the study of the histology of the pancreas (Bensley).

[3] This subject will be treated in a separate communication.

The principles underlying intravitam staining of zymogenic granules, which proved so useful in the study of the pancreas, should also afford us a means of determining conclusively whether or not trypsin or the products of the acinar cell activity can pass inward into the blood stream and exert the functions which we ascribe to them.

Injection of dyes has an added advantage in that it gives direct information of the vascular distribution of the part injected. In this manner we found that the circulatory supply of the pancreas in the cat is very variable.

Our experiments indicate (see protocol 7) that the injection of azine dyes (safranin, janus green and neutral red) into the pancreas via one of the arteries in which the pancreas alone is involved, does not cause any hyperglycemia or glycosuria. On the other hand, when the arterial distribution is such that the injection of the dye involves not only the pancreas but other organs as well, such as the pylorus, duodenum and mesentery, glycosuria may develop.

How are we to interpret these results? The difference between them must be due to a difference in the distribution of the dyes. When the pancreas alone is involved the dye becomes fixed in the acini and is kept from reaching the portal circulation, for a time at least, whereas when the other organs are involved some of it passes promptly into the portal circulation, ultimately reaching the liver, causing a glycogenolysis and sugar mobilization.

In order to test this hypothesis we injected the dyes into the portal vein directly (see protocol 8) and found that in each instance a glycosuria developed. We encountered but one exception to this, and that was in an animal with a very large cirrhotic liver. This exception was a most fortunate one in that it emphasized the rôle of the liver in the causation of hyperglycemia and glycosuria. This phase of the subject will be treated at another time.

Thus we have ascertained the following points:

1. That the injection of trypsin or saline or even insulin into the pancreatic circulation, however restricted the area, lead to a hyperglycemia and glycosuria.

2. Injection of trypsin into the portal circulation produces the same result, but the injection of saline or insulin does not.

3. Injection of an azine dye into the pancreas via its arterial supply does not cause a glycosuria provided the perfusion does not extend to adjacent or contiguous organs which permit ultimately some of the dye to pass into the portal stream. Injection of these dyes directly into the portal circulation causes a glycosuria. The azine dyes, as previously stated, have the power of staining and fixing the zymogenic granules and hence of combining with the trypsin which they contain.

Marston (36), quoting Cowdry, stated that janus green for example becomes firmly attached to the zymogenic granules in such a way that even after the bound dye becomes reduced and decolorized (thus becoming a leuco dye) the granules cannot again be stained by fresh dye.

If we were to ascribe the glycosuria which results from infusion of trypsin, saline or insulin into the pancreatic artery to a toxic action and damage of the islands of Langerhans, then we would surely expect the infusion of such agents as azine dyes to be productive of the same results.

When insulin is added to trypsin at a pH similar to that of the blood, the insulin becomes inactivated. If safranin or any other azine dye is first added to the trypsin and finally the insulin, no inactivation of the latter takes place.

If we correlate the above observations with the fact that azine dyes do not produce a glycosuria, we arrive at an explanation for the cause underlying this phenomenon. These dyes combine with the zymogenic granules of the acini and hinder the trypsin from entering the blood stream of the pancreas, thereby preventing on the one hand its action on the internal secretion, and, on the other, its access to the portal circulation.

That this conclusion is fully justified is further supported by additional experiments in which mixtures of insulin and azine dyes were used for perfusion (see protocol 9).

In this set of experiments, as stated, mixtures of insulin and azine dyes were injected into the pancreatic arteries. It will be recalled that infusion of insulin alone into the pancreatic arteries results in glycosuria. In contrast to this, however, are the results obtained when insulin mixed with azine dyes is infused. In these experiments no glycosuria developed. In other words, the presence of the azine dye in the infusion fluid protects the insulin (as in vitro) from the action of mobilized trypsin. The azine compound binds the trypsin in situ and allows the insulin to pass on unaffected through the pancreatic circulation into the portal system. It will be recalled also that insulin infused into the portal system does not cause a glycosuria.

As further proof of the contention that the hyperglycemia and glycosuria which follow the infusion of certain substances into the pancreatic circulation is the result of the mobilization of some substance (trypsin) present in the pancreas, and not to any injury to the pancreas, we have employed hydrazine sulphate for such infusions and found that no glycosuria results therefrom (see protocol 10). Hydrazine sulphate, as is well known, is a strong protoplasmic poison. Perfusion of the pancreas with a solution of this substance in sufficient concentration causes almost immediate destruction of the perfused part of the organ, yet no glycosuria develops. It seems reasonably certain that injury of the pancreas or any part of it, even to the point of necrosis (for that is the immediate effect of strong hydrazine solution), does not lead to glycosuria.

## CONCLUSIONS

The facts elicited point to the following conclusions:

1. No definite barrier exists between the structures which produce the internal secretion and those concerned with the external secretion of the pancreas.

2. The blood capillaries which supply the islands of Langerhans also supply the pancreatic acini.

3. The venous capillaries and large venules and veins which carry the internal secretion from the islands of Langerhans also drain the acini of active and effete products of metabolism. In other words, only one blood vascular system supplies both types of secreting glands of the pancreas.

4. Under certain conditions the blood capillaries become permeable to the secretory products of the acini, more particularly trypsin. Under these conditions trypsin becomes a constituent of the internal secretion.

5. The passage of trypsin into the blood stream of the pancreas has a twofold effect: namely, it promptly neutralizes whatever insulin it may come in contact with, and by passing into the portal circulation causes a glycogenolysis in the liver with a consequent hyperglycemia and glycosuria.

6. The fact that perfusion of the pancreas with insulin does not produce insulin effects but, on the contrary, leads to a glycosuria, indicates the possibility that the internal flow of trypsin may be part of a mechanism which regulates the supply and activity of the internal secretion of the pancreas. If over-activity of the islands of Langerhans can be compared with the process of infusion of free insulin into the pancreatic artery, then an excessive production of insulin, if neutralized by trypsin, would tend to produce a glycosuria rather than a hypoglycemia and insulinism.

7. Azine dyes and other azine substances combine with trypsin. The dyes stain and combine with the zymogenic granules of the acinar cells. When such substances are infused into the pancreatic vessels, no glycosuria results. On the other hand these substances do produce glycosuria if they are injected into the portal vein or if they reach the portal circulation via organs other than the pancreas.

8. Comparing the effect of perfusion of the pancreas with the aforesaid dyes with that of saline, insulin and trypsin, the conclusion is reached that the controlling factor in the activity of insulin is trypsin.

9. Injection of insulin mixed with azine dyes into the pancreatic artery does not cause glycosuria. The reason for this phenomenon seems to lie in the fact that these dyes fix the trypsin and prevent its mobilization into the pancreatic blood stream, thus hindering its effect on insulin.

10. Injury of the pancreas does not per se cause a glycosuria unless it creates an actual deficiency of insulin. Injury to part of the pancreas does not cause such a deficiency.

While a simple transient hyperglycemia and glycosuria cannot be designated as a diabetes, nevertheless if such a hyperglycemia and glycosuria is of pancreatic origin, it undoubtedly represents the same type of disorder that underlies a true pancreatic diabetes. Clinical experience teaches that many cases of diabetes may and often do begin as transient glycosurias. If we can use the evidence adduced in these experiments, we would say that the mechanism producing diabetes is comprised of two stages, the first consisting of a simple glycogenolysis and sugar mobilization, and the second in a diminished sugar utilization. The first stage can arise from the passage of trypsin into the liver by the portal circulation; the second from a deficiency of insulin caused by a decreased production, or what is probably more common, from a neutralization or inactivation of insulin by trypsin.

Work is in progress on animals with atrophied glands and animals with Eck fistulae in which the results obtained are being submitted to further proof.

The hypothesis developed in this study concerning the pathogenesis of diabetes suggests a method of treatment of the disease directed at the underlying cause. Such a prospect seems to be enhanced by the striking effects produced by the injection of azine dyes into the pancreatic circulation. This problem is under investigation at the present time.

We wish to acknowledge our indebtedness to the Eli Lilly Co. for their generous supply of iso-amyl-ethyl-barbituric acid (Amytal).

*Protocol 1.* Testing the effect of injection of trypsin into the pancreatico- duodenal artery.*

Experiment 1. (P-80). 9/2/24. Female cat, weight 3.2 kgm. Iso-amyl-ethyl-barbituric acid anesthesia. Laparotomy and 2 cc. trypsin injected into the pancreatico-duodenal artery. Abdomen closed in two layers.

*Observations*

| DATE | TIME | BLOOD SUGAR† | REMARKS |
|---|---|---|---|
| | | *per cent* | |
| 9/2/24 | 10:15 a.m. | 0.102 | 20 minutes after anesthesia, and 3 to 4 minutes after incision |
| | 10:20 | | Injected 2 cc. trypsin into artery |
| | 10:35 | 0.162 | After completion of operation |
| | 12:35 p.m. | 0.190 | |
| | 1:50 | 0.135 | |
| | 3:30 | 0.160 | |
| | 9:30 | 0.150 | |
| 9/3/24 | 1:30 p.m. | 0.145 | Animal has not eaten since operation |
| 9/4/24 | | | Animal eats meat; 210 cc. urine; sugar present: 0.5 per cent |
| 9/5/24 | | | Animal well; 30 cc. urine; sugar present; 0.6 per cent |

Experiment 2. (P-82). 9/2/24. Male cat, weight 3.5 kgm. Iso-amyl-ethyl-barbituric acid anesthesia. Laparotomy, 2 cc. trypsin injected into the pancreatico-duodenal artery. Wound closed in two layers.

*Observations*

| DATE | TIME | BLOOD SUGAR | REMARKS |
|---|---|---|---|
| | | *per cent* | |
| 9/2/24 | 10:45 a.m. | 0.110 | ¾ hour after anesthesia, and 5 minutes after incision |
| | 10:50 | | Trypsin injected |
| | 11:05 | 0.160 | 5 minutes after completion of operation |
| | 12:35 p.m. | 0.120 | |
| | 1:55 | 0.162 | |
| | 3:15 | | Voided 15 cc. urine. Sugar present; 0.6 per cent |
| | 3:40 | 0.165 | |
| | 9:05 | 0.164 | |
| 9/3/24 | 9:00 a.m. | | 28 cc. urine, sugar present; 0.6 per cent |
| | 1:30 p.m. | 0.160 | Animal not fed since operation |
| 9/4/24 | 9:00 a.m. | | Animal robust, eats meat. 45 cc. urine, sugar present; 0.5 per cent |
| 9/5/24 | 9:00 a.m. | | 60 cc. urine; sugar: faint trace present |

Comment: These experiments show that the infusion of trypsin into the pancreatico-duodenal artery causes a hyperglycemia and glycosuria which last for some time.

* These experiments are taken from a group of 9 similar operations.

† The blood sugar determinations were made by the Epstein modification of the Lewis-Benedict method.

*Protocol 2.* Testing the effect of injection of normal saline into the pancreatico-duodenal artery.*

Experiment 1. (P-84). 9/4/24. Male cat, weight 2.5 kgm. Iso-amyl-ethyl-barbituric acid anesthesia. Laparotomy and 2 cc. saline injected into the pancreatico-duodenal artery. Abdomen closed in two layers.

*Observations*

| DATE | TIME | BLOOD SUGAR | REMARKS |
|---|---|---|---|
| | | *per cent* | |
| 9/4/24 | 10:25 a.m. | 0.116 | ½ hour after anesthesia, and few minutes after incision and dissection |
| | 10:30 | | Injection of saline into artery |
| | 10:45 | 0.174 | 5 minutes after completion of operation |
| | 1:05 p.m. | 0.172 | |
| | 2:15 p.m. | 0.172 | |
| | 4:00 p.m. | 0.150 | |
| | 9:00 p.m. | 0.110 | |
| 9/5/24 | 1:00 p.m. | | 70 cc. urine; sugar present: 0.7 per cent<br>Animal has been eating meat |
| | 3:00 p.m. | 0 160 | |
| 9/6/24 | 4:00 p.m. | | 43 cc. urine; sugar present: 0.8 per cent<br>Animal eating well |

Comment: This experiment shows that the infusion of saline into the pancreatico-duodenal artery causes a hyperglycemia and glycosuria which lasts for some time.

* This experiment is one of three similar operations.

*Protocol 3.* Testing the effect of injection of insulin into the pancreatico-duodenal artery.**

Experiment 1. (P-83). 9/2/24. Female cat, weight 2.6 kgm. Iso-amyl-ethyl-barbituric acid anesthesia. Laparotomy, 1 cc. (20 units) insulin, lot #77547-751368, neutralized and volume made up to 2 cc. with saline,† injected into the pancreatico-duodenal artery.

*Observations*

| DATE | TIME | BLOOD SUGAR | REMARKS |
|---|---|---|---|
| | | *per cent* | |
| 9/2/24 | 11:10 a.m. | 0.100 | 1 hour after anesthesia, and 2 to 3 minutes after abdominal incision |
| | 11:30 | 0.148 | 5 minutes after completion of operation |
| | 12:40 p.m. | 0.120 | |
| | 2:00 | 0.105 | |
| | 3:50 | 0.120 | |
| | 9:10 | 0.156 | |
| 9/3/24 | 1:30 p.m. | 0.140 | Animal robust; has not eaten since operation |
| 9/4/24 | | | 77 cc. urine; sugar present: 0.55 per cent<br>Animal eating meat |

Experiment 2. (P-15). 5/15/24. Male cat, weight 3.15 kgm. Ether anesthesia. Laparotomy, pancreatico-duodenal artery exposed and 1 cc. (20 units) insulin, lot #77186–751365 + saline to make volume up to 2 cc. injected. There was very little manipulation and practically no bleeding. Wound sewed up in two layers.

*Observations*

5/16/24 No urine; cat well
5/17/24 100 cc. urine; sugar present: 0.5 per cent
5/18/24 100 cc. urine; sugar present: 0.3 per cent
Animal robust and eating well
5/19/24 110 cc. urine; sugar 0
5/20/24 115 cc. urine; sugar present: 0.3 per cent
5/21/24 320 cc. urine; sugar: questionable reaction

Experiment 3. (P-20). 5/22/24. Male cat weight 3.0 kgm. Iso-amyl-ethyl-barbituric acid anesthesia. Laparotomy, 1.5 cc. (30 units) insulin lot #77186–751365 + saline to make volume up to 2 cc. injected into the pancreatico-duodenal artery. Wound closed.

*Observations*

5/22/24 30 cc. urine voided 3 hours after operation. Sugar present: 0.6 per cent
5/23/24 6 cc. urine; sugar present: 0.7 per cent
5/24/24 No urine. Cat well
5/25/24 Urine mixed with stool; unfit for examination
5/26/24 140 cc. urine. Sugar 0

Comment: All the experiments show that the injection of insulin into the pancreatico-duodenal artery causes a glycosuria. The first experiment shows the effect on the blood sugar. It will be seen that the sugar curve is somewhat different from that obtained with the trypsin and saline injections. After a preliminary rise there is a slight fall which is followed by a rise again. The intermediate fall is probably due to the passage of some of the insulin via the duodenal branch of the artery, thus flowing into the portal vein without coming in contact with the pancreatic tissues. It is noteworthy that insulin introduced into the pancreatico-duodenal artery does *not* cause a hypoglycemia.

** These experiments are taken from a group of 7 similar operations.

† The object of adding saline was to make the volume of fluid injected uniform with that of the preceding experiments.

*Protocol 4.* Testing the effect of injection of trypsin into the portal vein.*

Experiment 1. (P-86). 9/4/24. Female cat, weight 3.1 kgm. Iso-amyl-ethyl-barbituric acid anesthesia. Laparotomy and 2 cc. trypsin injected into the portal vein. Wound closed in two layers; operation completed in 15 minutes.

*Observations*

| DATE | TIME | BLOOD SUGAR | REMARKS |
|---|---|---|---|
| | | *per cent* | |
| 9/4/24 | 11:10 a.m. | 0.108 | ¾ hour after anesthesia, and directly after abdominal incision |
| | 11:12 | | Trypsin injected into portal |
| | 11:25 | 0.180 | 5 minutes after completion of operation |
| | 1:15 p.m. | 0.140 | |
| | 2:25 | 0.174 | |
| | 4:00 | 0.200 | |
| | 9:00 | 0.170 | |
| 9/5/24 | 3:30 p.m. | 0.140 | Animal well; no urine |
| 9/6/24 | 4:00 p.m. | | 128 cc. amber urine; sugar present: 0.4 per cent |

Comment: This experiment shows that the infusion of trypsin into the portal vein causes a hyperglycemia and glycosuria.

* This experiment is taken from a group of 4 similar operations.

*Protocol 5.* Testing the effect of injection of insulin into the portal vein.**

Experiment 1. (P-56). 6/21/24. Male cat, weight 2.6 kgm. Iso-amyl-ethyl-barbituric acid anesthesia. Laparotomy, 1 cc. (20 units) insulin, lot #77186-751365, + 1 cc. saline injected into the portal vein. Operation completed in 10 minutes.

*Observations*

6/22/24 No urine
6/23/24 87 cc. urine; sugar 0
6/24/24 50 cc. urine; sugar 0

Experiment 2. (P-76). 8/21/24. Female cat, weight 2.7 kgm. Iso-amyl-ethyl barbituric acid anesthesia. Laparotomy, 1 cc. (20 units) insulin, lot #77547-751368, + 1 cc. saline injected into portal vein. Wound closed in three layers.

*Observations*

| DATE | TIME | BLOOD SUGAR | REMARKS |
|---|---|---|---|
| | | *per cent* | |
| 8/21/24 | 10:40 a.m. | 0.131 | Before injection |
| | 10:48 | | Injection of insulin into portal vein |
| | 11:50 | 0.075 | |
| | 1:45 p.m. | 0.055 | |
| | 4:15 | 0.065 | |
| 8/22/24 | 9:00 a.m. | 0.068 | Animal still drowsy; 23 cc. urine; sugar 0 |
| 8/23/24 | | | 39 cc. urine; sugar 0 |

Comment: These experiments show that the infusion of insulin into the portal vein results in the reduction of the blood sugar. Insulin so introduced does not cause a glycosuria.

** These experiments represent 2 of 5 similar operations.

*Protocol 6.* Testing the effect of injection of saline into the portal vein.*

Experiment 1. (P-67). 6/24/24. Female cat, weight 2.5 kgm. Iso-amyl-ethyl-barbituric acid anesthesia. Laparotomy, 2 cc. saline injected into portal vein. Operation completed in 12 minutes.

*Observations*

6/25/24 70 cc. urine; sugar 0. Cat eating meat.
6/26/24 No urine.
6/27/24 35 cc. urine; sugar 0. Cat robust.

Experiment 2. (P-74). 8/21/24. Female cat, weight 3.7 kgm. Iso-amyl ethyl-barbituric acid anesthesia. Laporatomy, 2 cc. saline injected into porta vein. Wound closed in 3 layers.

*Observations*

| DATE | TIME | BLOOD SUGAR | REMARKS |
|---|---|---|---|
| | | *per cent* | |
| 8/21/24 | 10:20 a.m. | 0.115 | Before injection |
| | 10:24 | | Saline injected into portal vein |
| | 10:30 | 0.130 | |
| | 1:35 p.m. | 0.120 | |
| | 3:30 | | 30 cc. urine; sugar 0 |
| | 4:05 | 0.140 | |
| 8/22/24 | 9:00 a.m. | 0.084 | 49 cc. urine; sugar 0 |
| | | | Animal robust and eating |

Comment: These experiments show that the infusion of saline into the portal vein causes an insignificant rise in the blood sugar. It does not produce any glycosuria.

* These experiments represent 2 of 4 similar operations.

*Protocol 7.* Testing the effect of injection of azine dyes (safranin, janus green and neutral red) into the pancreatico-duodenal artery.**

Experiment 1. (P-29). Safranin. 6/3/24. Male cat, weight 2.1 kgm. Iso-amyl-ethyl-barbituric acid anesthesia. Laparotomy, 2 cc. safranin (1 per cent) injected into the pancreatico-duodenal artery. Wound closed in two layers.

*Observations*

| DATE | TIME | BLOOD SUGAR | REMARKS |
|---|---|---|---|
| | | *per cent* | |
| 6/3/24 | 10:10 a.m. | 0.150 | Before injection |
| | 10:15 | | Injection of dye |
| | 12:10 p.m. | 0.125 | |
| | 2:15 | 0.105 | |
| | 9:30 | 0.102 | 28 cc. urine (colored red); sugar 0; albumin 0 |
| 6/4/24 | 10:00 a.m. | | 40 cc. urine (colored red); sugar 0; albumin 0 |
| | 12:00 noon | 0.125 | |
| 6/5/24 | 9:00 a.m. | | 40 cc. urine (colored deep red); sugar 0 |
| 6/6/24 | 9:00 a.m. | | 80 cc. urine (colored red); sugar 0 |

**This experiment is one of 4 similar operations.

Experiment 2. (P-27). Janus green. 5/29/24. Female cat, weight 2.5 kgm. Iso-amyl-ethyl-barbituric acid anesthesia. Laparotomy, 2 cc. janus green (1 per cent) injected into pancreatico-duodenal artery. Operation completed in 15 minutes.

*Observations*

| DATE | TIME | BLOOD SUGAR | REMARKS |
|---|---|---|---|
| | | *per cent* | |
| 5/29/24 | 10:00 a.m. | 0.103 | Before injection |
| | 10:00 | | Injection of dye |
| | 10:20 | 0.120 | |
| | 4:00 p.m. | 0.140 | |
| 5/30/24 | 9:00 a.m. | | 27 cc. urine (amber color); sugar 0 |
| | 10:30 a.m. | | Animal moribund; killed; 2 cc. urine (amber color) aspirated from bladder; sugar 0 |

**This experiment is one of 5 similar experiments.

Experiment 3. (P-45). Neutral red. 6/12/24 Male cat, weight 2.5 kgm. Iso-amyl-ethyl-barbituric acid anesthesia. Laparotomy, 2 cc. neutral red (1 per cent) injected into the pancreatico-duodenal artery. Operation completed in 15 minutes.

*Observations*

6/13/24. No urine, animal robust.
6/14/24 9 a.m. 90 cc. urine (colored pink); sugar 0
4 p.m. 63 cc. urine (amber color); sugar 0
**This experiment represents one of 2 similar operations.

Comment: These experiments show that the infusion of azine dyes into the pancreatico-duodenal artery does not cause either a hyperglycemia or a glycosuria. In some of the experiments with azine dyes glycosuria did develop; in these experiments it was observed that the infusions involved a number of adjacent structures such as the pylorus, duodenum and its mesentery.

*Protocol 8.* Testing the effect of injection of azine dyes (safranin and janus green) into the portal vein.*

Experiment 1. (P-87). 9/4/24. Male cat, weight 3.4 kgm. Iso-amyl-ethyl-barbituric acid anesthesia. Laparotomy, 2 cc. of safranin (1 per cent solution) injected into the portal vein. Abdomen closed in two layers.

*Observations*

| DATE | TIME | BLOOD SUGAR | REMARKS |
|---|---|---|---|
| | | *per cent* | |
| 9/4/24 | 10:25 a.m. | 0.112 | ½ hour after anesthesia and at time of incision |
| | 10:30 | | Safranin injected into portal |
| | 10:40 | 0.176 | 5 minutes after completion of operation |
| | 1:20 p.m. | 0.150 | |
| | 2:30 | 0.150 | |
| | 4:15 | 0.150 | |
| | 9:00 | 0.150 | |
| 9/5/24 | 1:00 p.m. | | 56 cc. dark red urine; sugar present: 0.66 per cent |
| | 3:30 | 0.160 | |
| 9/6/24 | 4:00 p.m. | | 55 cc. urine (colored red); sugar present: 0.5 per cent |

Experiment 2. (P-28.) 5/29/24. Male cat, weight 2.85 kgm. Iso-amyl-ethyl-barbituric acid anesthesia. Laparotomy, 2 cc. janus green (1 per cent solution) injected into the portal vein. Wound sewed up and operation completed in 15 minutes.

*Observations*

| DATE | TIME | BLOOD SUGAR | REMARKS |
|---|---|---|---|
| | | *per cent* | |
| 5/29/24 | 10:45 a.m. | 0.133 | After administration of anesthesia |
| | 11:00 | | Janus green injected into portal |
| | 4:00 p.m. | 0.376 | |
| | 10:30 | 0.250 | |
| 5/30/24 | 9:00 a.m. | | Animal moribund; 18 cc. urine (amber color); sugar present: 5.0 per cent |
| | 10:30 | | Animal killed; 34 cc. urine (amber color); sugar present: 1.7 per cent albumin 0 |

Comment: These experiments show that the infusion of the azine dyes into the portal vein produce both a hyperglycemia and a glycosuria.

* Experiment 1 is one of three similar operations. Experiment 2 is one of two similar operations.

*Protocol 9.* Testing the effect of injection of mixtures of insulin and azine dyes (safranin, janus green, indulin and neutral red) into the pancreatico-duodenal artery.

Experiment 1. (P-85). 9/4/24. Insulin + safranin. Male cat, weight 2.3 kgm. Iso-amyl-ethyl-barbituric acid anesthesia. Laparotomy, and 1 cc. (20 units) insulin, lot #77547-751368 + 1 cc. safranin (1 per cent) injected into the pancreatico-duodenal artery. Wound closed in two layers.

*Observations*

| DATE | TIME | BLOOD SUGAR | REMARKS |
|---|---|---|---|
| | | *per cent* | |
| 9/4/24 | 10:50 a.m. | 0.124 | Directly after incision |
| | 10:55 | | Insulin + safranin injected |
| | 11:05 | 0.140 | |
| | 1:10 p.m. | 0.064 | |
| | 2:20 | 0.055 | |
| | 4:15 | 0.058 | |
| | 9:00 p.m. | 0.070 | |
| 9/5/24 | 1:00 p.m. | | 42 cc. urine (colored red); sugar 0 |
| | 3:10 p.m. | 0.130 | Animal eating |
| 9/6/24 | 4:00 p.m. | | 78 cc. urine (colored pink); sugar 0 |

Experiment 2. (P-49). 6/17/24. Insulin + safranin. Male cat, weight 3.5 kgm. Iso-amyl-ethyl-barbituric acid anesthesia. Laparotomy and 1 cc. (20 units) insulin, lot #77125-751364, + 1 cc. safranin (1 per cent) injected into the pancreatico-duodenal artery. Wound closed in two layers.

*Observations*

6/18/24 No urine; animal appears sick.
6/19/24 No urine
6/20/24 160 cc. urine (dark amber); sugar 0; albumin 0

These experiments represent 2 of 4 similar operations.

Experiment 3. (P-54). 6/19/24. Insulin + janus green. Male cat, weight 3.4 kgm. Iso-amyl-ethyl-barbituric acid anesthesia. Laporatomy, 1 cc. (20 units) insulin, lot #77125-751364 + 1 cc. janus green (1 per cent) injected into the pancreatico-duodenal artery. Operation completed in 15 minutes.

*Observations*

6/20/24 41 cc. urine (amber color); sugar 0
6/21/24 No urine
6/22/24 95 cc. urine (amber color); sugar 0

This experiment represents one of 2 similar operations.

Experiment 4. (P-50). 6/17/24. Insulin + indulin. Female cat, weight 3 kgm. Iso-amyl-ethyl-barbituric acid anesthesia. Laporatomy, 1 cc. (20 units) insulin, lot #77125-751364 + 1 cc. indulin (1 per cent) injected into the pancreatico-duodenal artery. Wound closed in two layers.

*Observations*

6/18/24 Animal robust. No urine
6/19/24 9:00 a.m. 58 cc. urine (greenish amber color); sugar 0
11:00 a.m. 32 cc. urine (greenish amber color); sugar 0

This experiment represents one of 2 similar operations.

Experiment 5. (P-48). 6/17/24. Insulin + neutral red. Male cat, weight 2.5 kgm. Iso-amyl-ethyl-barbituric acid anesthesia. Laparotomy, 1 cc. (20 units) insulin, lot #77125-751364 + 1 cc. neutral red (1 per cent) injected into the pancreatico-duodenal artery. Wound closed in two layers.

*Observations*

6/17/24 Animal voided 35 cc. urine (colored pink) 3 hours after operation, sugar 0.
6/18/24 9:00 a.m. 30 cc. urine (colored pink); sugar 0
6/19/24 9:00 a.m. 33 cc. urine (colored pink); sugar 0

This experiment represents one of 4 similar operations.

Comment: These experiments show that the infusion of mixtures of insulin and azine dyes does not cause a glycosuria. The insulin under the above conditions produces its physiological effect on the blood sugar, causing a hypoglycemia.

*Protocol 10.* Testing the effect of injection of hydrazine sulphate into the pancreatico-duodenal artery.*

Experiment 1. (P-64). 6/24/24. Male cat, weight 2.5 kgm. Iso-amyl-ethyl-barbituric acid anesthesia. Laparotomy, 2 cc. of hydrazine sulphate (2 per cent injected into pancreatico-duodenal artery. Operation completed in 15 minutes.

*Observations*

6/25/24 Animal dead. 18 cc. urine (amber color) found in container; sugar 0

Experiment 2. (P-68). 6/26/24. Female cat, weight 3.9 kgm. Iso-amyl-ethyl-barbituric acid anesthesia. Laparotomy, 2 cc. hydrazine sulphate (2/10 per cent) injected into the pancreatico-duodenal artery. Operation completed in 10 minutes.

*Observations*

6/27/24 15 cc. urine (amber color); sugar 0
6/28/24 120 cc. urine (amber color); sugar 0

Comment: These experiments show that infusion of hydrazine sulphate into the pancreatico-duodenal artery does not cause a glycosuria. Blood sugar determinations were not made.

* These experiments represent 2 of 3 similar operations.

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# COMPARATIVE STUDIES ON THE EXCITABILITY OF THE FOREBRAIN

THEODORE KOPPÁNYI AND J. FRANK PEARCY

*From the Hull Physiological Laboratory of the University of Chicago*

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Whereas there has been a large amount of very accurate experimentation done upon the excitability of the forebrain of mammals, very little has been done on the lower vertebrate forms. Rogers (3), working on one of the lowest types of mammals, the opossum (Didelphys virginiana), found that there was a restricted area of the forebrain which was similar to the "motor cortex" of the higher mammals. Rogers found in the pigeon that stimulation of an area in the medial occipital region caused constriction of the pupil. Johnston (2) studied the excitability of the forebrain of the reptiles. He found that stimulation of the dorsal surface of the olfactory bulb caused retraction of the neck, extension of the legs, movement of the eyeball and eyelid; the dorsal surface of the pallium near the olfactory peduncle and lateral border of the pallium in the anterior one-half or two-thirds of the hemisphere caused movement of the eyes, jaw, neck, legs and tail; striatal area caused movement of all parts. No response was obtained from other portions of the surface of the forebrain. He thinks that the results from stimulation of the olfactory and striatal areas are due to spread of current. Thus it appears that a comma-shaped area involving the rostral and lateral borders of the general pallium may be regarded as a motor area in the turtle's pallium.

One is struck by the statement of Johnston that the responses were best obtained when the animals were deeply anesthetized, that as the degree of anesthesia was made lighter the responses were more widespread and irregular, suggestive of stimulation from spreading of the electrical current. As monopolar stimulation was used, this statement is more significant for in small animals one can readily ascertain the free spreading of the current when the forebrain is stimulated by this method. Furthermore, there is evidence that the portions of the nervous system most readily affected by anesthesia are the higher centers and would accordingly be the first to be eliminated by deepening the anesthesia.

Ivy (1) in discussing the relation of the forebrain to nystagmus, states, "The presence of a motor cortex in rabbit, cat, and dog is well recognized." Such an area has never been demonstrated in the cerebrum of the frog

and pigeon, while it has been alleged by Johnston for the turtle, which, however, is still a mooted question.

EXPERIMENTAL. *Fish.* We were supplied by large vigorous specimens of the gold fish (carassius auratus) by Mr. Parker and Mr. Young of the Lincoln Park Zoölogical Gardens. The bone was removed from the head of the fish overlying the forebrain. The fatty material over the hemispheres was removed. The fish was placed in water every few minutes so as to prevent asphyxia. It was held in a towel during the experimental work. The dorsal surface of the forebrain was stimulated with a faradic current just detectable to the tongue by the bipolar method. There resulted at once swimming movements of the tail. These movements were slow, regular, propeller-like. The ventral thoracic fins also showed rhythmical swimming movements. Increasing the strength of stimulation increased the force of the swimming movements but did not alter the character of these movements. The electrodes were removed and a probe was introduced into one hemisphere of the forebrain. The same typical swimming movements resulted. The forebrain was transected by a careful section with delicate cataract scissors just cephalad to the corpora bigemina. Stimulation of the forebrain was now quite without motor response. Exactly similar results were obtained upon numerous of these fish. Stimulation of the forebrain always gave these same slow rhythmical swimming movements, whereas stimulation of adjacent structures caused violent escaping movements.

*Newts.* The newt (Diemyctylus torosus) from Oregon was used for these experiments. The brain was exposed from above and the forebrain stimulated. The results in every case were negative. In none of these animals was there any well defined response to stimulation cephalad to the corpora bigemina.

*Frogs.* Numerous Rana pipiens and Rana catesbiana were used. The brain was exposed and the forebrain stimulated. In every case the results were negative. The experiments were done on animals in good condition.

*Turtles.* The brain was exposed as it was in the amphibia. The dura was removed. Bipolar stimulation of the cortex gave negative results. Very mild stimulation of the deeper portions of the forebrain, the corpus striatum, produced slow rhythmical swimming movements of all four legs. Increasing the strength of the stimulation increased the response in degree but did not alter it in character. Stimulation of the cerebral peduncles produced the same response and was elicited with a weaker current. Forebrain and peduncle stimulation was often followed by an extensor after discharge of the forelegs which lasted for one-eighth to one-half a minute depending upon the intensity and duration of the stimulus. Tremors of the hind legs were often observed after strong stimulation of the forebrain. The optic lobes were removed without obviously altering the response to

forebrain stimulation. The peduncles were carefully sectioned with delicate scissors. Stimulation of the forebrain was now without motor response. These experiments were repeated upon young, six-inch and fourteen-inch turtles with the same results.

*Alligators.* One alligator about eighteen inches long was used. Forebrain stimulation was without any regular motor response.

*Birds.* Several pigeons were used. The brain was exposed with the animals under ether. They were allowed to recover from the anesthesia before they were tested. Stimulation of the forebrain was without motor response. No response was obtainable except when the electrodes were placed near the cerebellum when violent movements resulted.

*Mammals.* White rats were used. The brain was exposed during ether anesthesia. The forebrain was explored with bipolar electrodes after the animals had partly recovered from the anesthesia. Stimulation of a small area (about 3 mm. in diameter) in the central part of the frontal lobe produced extension of the heterolateral hind leg. When the current was made a little stronger the heterolateral front leg was also extended. No other portion of the cortex produced definite motor responses.

Discussion. Previous to the present work the reptile was considered to be the lowest animal with excitable forebrain. We have placed the limit considerably lower (phylogenetically older). The teleost certainly has an excitable forebrain. At present we are unable to say whether the still older forms have an excitable forebrain as, for instance, the elasmobranchs, ganoids, etc. We purpose to determine this later. We conclude that the archipallium (forebrain) is excitable in certain fish (teleosts) and certain reptiles (turtles). The neopallium (cortex) is not excitable (as judged by skeletal response) except in mammals. Johnston claims that an excitable neopallium is present in reptiles. Our failure to obtain these results is perhaps explicable by the difference in experimental methods. Following the precedence of the work on fine localization on the cortex on large animals we began our work using the monopolar method of stimulation. We soon became suspicious of this method when great difficulty was experienced in preventing irregular responses from forebrain stimulation. These irregularities were due apparently to stimulation at some distance from the point of the electrode. The indifferent electrode was then placed in the head anterior to the excitation electrode with the result that these irregular movements were considerably lessened. Feeling that the monopolar method might be unsuitable for these smaller animals we tried the bipolar method. Two minute blood vessel suture needles with points about a millimeter apart were used for the electrodes. The results were quite convincing. The responses were accurately localized and free from the irregularities previously experienced. The monopolar method was consequently discarded as unsuitable for small animal work.

Jackson showed long ago that the cerebral cortex is the first to be affected by narcosis. A large mass of evidence has accumulated in support of this view whereas there has been no convincing evidence to the contrary. One may be skeptical of results from neopallium stimulation accomplished under deep narcosis not obtainable under light anesthesia. Increasing narcosis would not hold in abeyance non-cortical responses so as to make more obvious the cortical ones, as Johnston seems to have imagined, for certainly the cortical responses would be decreased most, leaving the subcortical responses less affected. Having used non-narcotized animals and delicate bipolar electrodes with a faradic current just detectable to the tongue and having obtained by stimulation of the archipallium regular, rhythmical movements similar to those used by the animal in slow locomotion and no response from neopallium stimulation until strong currents were used (and these were vigorous, irregular movements), it appears to us that there is no escaping the conclusion that skeletal motor responses are obtainable from the archipallium of the turtle and are not obtained from the neopallium.

It appears at a first glance to be singular that fish have an excitable forebrain and amphibia do not, that turtles have and alligators do not, that birds do not, whereas mammals do. But consideration of the phylogenetic relations may help to explain these apparent contradictions.

Beginning with the phylogeny of the vertebrates, according to Hatschek (4), we believe that the primitive fish forms had a forebrain which was inexcitable. We have been unable to work on the ganoids or elasmobranchs. These may be negative but even if positive it is quite likely that the more primitive ancient forms were negative. The amphibia rising from these primitive fish forms retained the inexcitable forebrain.

The crocodilia, which also have the inexcitable forebrain, may have developed from the amphibia. It is believed by Hatschek and other morphologists and zoölogists that the birds are most closely related to the crocodilia. It is therefore to be expected that they should also possess an inexcitable forebrain. But from the primitive fish forms arose other forms with excitable forebrains; of these we have at present the teleosts (possibly others). The present conception among zoölogists is that the main line of reptiles arose from the fish independently of amphibia. These reptiles, possessing excitable forebrain, arose from those fish forms possessing such an excitable forebrain. Among these are the turtles. *The turtles are considered by Hatschek to be the nearest relative of the mammals.* It was therefore to be anticipated that both should possess excitable forebrain.

We thus offer this physiological evidence in confirmation of the morphological evidence of the relationship of the phyla and inheritance. We contemplate further physiological experiments to test the validity of the above classification.

SUMMARY

1. Definite skeletal motor responses are produced by proper electrical stimulation of the forebrain in the bony fishes and turtles, but not in newts, frogs, alligators and birds. This physiological difference supports Hatschek's phylogenetic scheme.

2. The rat has a definite motor cortex in the frontal lobe of the cerebrum analogous to the higher mammals.

We desire to express our hearty gratitude to Doctor Carlson for his help and criticism while this work was in process.

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# FURTHER STUDIES ON EYE TRANSPLANTATION IN THE SPOTTED RAT

THEODORE KOPPÁNYI AND CLYDE BAKER

*From the Hull Physiological Laboratory of the University of Chicago*

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It has been shown by Koppányi (1), (2), (3) and by Kolmer (4) that the eyes of various cold- and warm-blooded vertebrates can be extirpated and transplanted back in the eye socket, and the recovery of certain functions of such transplanted eyeball obtained.

It has been shown by several investigators (von Frisch, Przibram, Koppányi) that blinded fishes and blinded amphibians lose their ability of color adaptation and the color of the skin becomes uniformly dark, in fact nearly black. These observations suggested that perhaps the eye may control the color adaptation of the skin in many cold-blooded vertebrates. Mast (5) has reported that it is sufficient to hold a yellow or violet colored paper before the eyes of the flounder (Paralichthys and Ancylopsetta) to change the skin color approximately to that of the paper.

This observation shows that the eye is concerned with the color adaptation but it is still an open question whether the phenomenon is a direct visual function or the result of some unknown endocrine function.

Koppányi transplated eyes into the neck region on metamorphosed Bombinator igneus Rösel, after a modified method of Uhlenhuth (6). Koppányi corroborated the results of Uhlenhuth. The gross appearance of these transplanted eyes was normal. When the eye was successfully transplanted into the neck region, Koppányi was unable to produce the reappearance of the color adaptation and the disappearance of the blind color in these blinded amphibians. Therefore, it seems that the control of the color adaptation by a local endocrine-like function of the eyes is excluded.

In a second series of experiments on adult Bombinator the eyeball was excised and replanted into the eye socket. These transplanted eyes retained their normal appearance. Eight weeks or later after the eye transplantation the normal color of the skin of these animals reappeared. This fact proves that the eye controls the color adaptation of the skin by its visual function. The animals showed normal phototaxis and were able to hunt and capture flies. Histological examination of these transplanted eyes by Kolmer showed the presence of all the layers in the retina and re-

generation of the optic fibers. Thus, the study of the mechanism of color adaptation in cold-blooded vertebrates led to successful transplantation of the entire eyeball.

Koppányi extended the eye transplantations to the Norwegian rat (Epimys norvegicus Erxl.) and showed that the transplanted eyeball of the spotted rat recovers several functions which are normal for the same animal. Kolmer has shown that in the most successful cases the transplanted eyeball contains rods and cones in the retina and nerve fibers pass from the papillae by the optic nerve to the chiasma. G. Guist (7) repeated Koppányi's experiments and reported mainly negative results.

The experiments here reported were begun in February, 1924. We made autoplastic eye grafts in the spotted rat; that is, we replanted the enucleated eyeball back into the same orbit by the following method: Under ether anesthesia the head, including the surrounding environment of the orbit, was shaved, the soap and hair removed with hot water, and the field washed with alcohol and picric acid. The animal (except the head) and the animal holder was covered with sterile towels. The technique of the extirpation and replantation of the eyeball was essentially that described by Koppányi. The essential point in the enucleation, is the retention of a large piece of the conjunctiva but no muscles and nerves attached to the eye ball. The extirpated eyeball is handled by the attached conjunctiva. After replacing the eyeball in the socket the attached conjunctiva is removed and the lids closed with one or two stitches. In all animals worked on both eyes were thus transplanted.

We waited until the sutured eyelids separated of their own accord and when the eyelids were opened, we were able to begin our observation on the grafts. We operated upon 25 spotted rats. The first spotted rats were operated upon February 13, 1924, and the last were operated upon May 8. In 20 of these rats the grafted eyeballs failed to take. The eyes underwent the process of panophthalmia. We noticed first in such panophthalmic eyes a buphthalmus and then a perforation of the cornea. After two weeks we saw in those orbits only the sclera and the opaque lens. The sclera, increased in thickness, stays in nearly every case.

In five rats the transplanted eyes showed varying degrees of return of function. In two of these animals only the corneal reflex returned. In two animals we noted the corneal reflex and mobility of the eyeballs. In one rat, the most successful transplant, the pupillary reaction to light and some biological reactions indicating vision could also be observed. When the eyelids are opened 2 or 3 weeks after the transplantation, the cornea may be opaque or already transparent. Sometimes the cornea is cherry red. In such cornea a few days later we see, on the surface and deeply, single blood vessels, which disappear completely about two weeks later. We know that the cornea under normal physiological conditions

never undergoes vascularization. But in human pathology we know a disease—parenchymatous keratitis—in which the cornea shows similar vascularization. Although the perfect recovery of transparency must be rare in this disease, in the transplanted eyes the recovery of transparency is complete and we can see the minute vessels in the cornea when it is already cleared up. The disappearance of these minute vessels takes no more than two to four weeks in the transplanted eyes of the rat, while Nettleship and Hirschberg have observed on human eyes vascularization even 15 years after the attack of this disease. We assume that in the human cases and probably in the transplanted corneas that these minute vessels come from the ciliary vessels. We call this phenomenon the experimental vascularization of the cornea.

The rats operated upon March 28 showed complete degeneration of the right eye; the rat operated upon March 29 showed complete degeneration of the left eye. The remaining eye in each case healed up but showed concentric shrinkage. The corneal reflex returned, the motility of the eyeballs was doubtful. There was also iridocyclitis and an atrophic iris present. The cornea was clear. Although no normal pupil was present we were able to see the opaque lens. The animals were killed June 17, 1294, and both eyes were preserved in 10 per cent formalin.

A second type of results was shown by the animals that we operated upon February 13, 1924, and February 28, 1924. The eyes with respect to size and form were normal in appearance, the cornea clear, corneal reflex present but the lens was opaque. In one case the iris was not continuous. The pupils were round with jagged edges and immobile; the cause of this immobility was a staphyloma, the iris being connected in some places to the cornea. Mobility of the eyeballs was present. We were unable to differentiate these eye movements from the eye movements of the normal rats. This fact proves at least some return of function of the eye muscles and the oculomotor nerve. Because of the small size of the midbrain in the rat, we have so far failed in the technic of faradic stimulation of the oculomotor nuclei in these operated rats. These two animals were killed and preserved in 10 per cent formalin on June 17, 1924.

The most successful eye grafts were in the animal operated upon March 24, 1924.

We have mentioned that after the reimplantation we sewed the eyelids together, then waited until the sutures dropped out, allowing the eyelids to open. The sewing together of the eyelids saves the cornea from a dry mummification and against mechanical injuries. In this case the eyelids held together for over two weeks, and when the eyelids opened we were able to see the iris and pupil in both eyes. The iris and pupil of the right eye were regular, those of the left eye irregular. The pupils on both sides

were immobile. The pupillary reactions were tested by strong sunlight but neither Doctor Carlson, Doctor Lim or ourselves were able to notice any iris movements. Two weeks later we noticed movements of the pupil. It was probably a light reflex but there was some doubt that it might not have been so. We noticed physiological mobility of the eyeballs and corneal reflexes both in the right and left eyes, although the left eye was somewhat shrunken.

The animal developed pneumonia, and on the animal in this condition a careful examination of the pupil reactions was made on May 27. Doctor Ivy aided us in this examination and we decided that the pupil constricted under the influence of light and dilated again when the animal was placed in the dark. We also tried some biological tests for vision before the animal was killed with an injection of 10 per cent formalin into the heart to secure perfect preservation of the eyes. Afterwards the head was cut off and put in formalin. Doctor Carlson dissected the fixed material on June 17 and we noticed that the optic nerves on both sides were connected with the center of the eyeball. The optic tract of the left shrunken eyeball was smaller than the optic tract of the right.

In the case of the two rats with eye transplantations not done in this laboratory, in one animal the right eye became completely absorbed but the left eye was of normal appearance. The reaction of the pupil to light was present in this eye and the pupil dilated under asphyxia. In the second rat both eyes were good, the right eye pupil was enlarged and slightly irregular. The corneal and pupil reflexes were present in both eyes.

The Claude Bernard experiment that stimulation of the cervical sympathetic causes dilatation of the pupil, failed in both animals, but during the stimulation we noticed movements of the eyeballs.

Normal rats show leukophobia, i.e., going away from strong light, while blind rats do not react to light in this manner. Therefore a test for negative phototaxis was used as an index of vision in rats with transplanted eyes.

In the early tests we used a wooden box containing one light and one dark compartment with a hole in the partition separating the two compartments. Doctor Carlson suggested an improvement in this apparatus, in which we were able with a hinged cover to make sometimes the left and sometimes the right compartment dark and therefore the light test could be repeated many times.

Blind rats are unable to discriminate between the light and the dark chambers of the box. They walk around in either compartment irrespective of the light conditions. Normal spotted rats remain only 7 to 15 seconds in the light compartment before passing through the opening to the dark compartment. Three rats with transplanted eyes reacted in this test like normal rats.

The modified Waugh test was also used for testing the visual functions of the transplanted eyeball. The animal is placed on a platform about six inches square and about one foot from the table. Blind rats do not *jump* down to the table from the platform. Sometimes the blind rats *crawl* down from the platform clinging to the iron rod on which the platform rests, probably guided by the sense of touch. Normal rats and the three spotted rats with transplanted eyes *jumped down* from the platform. The higher the platform from the table the longer the animal hesitates before it jumps down. We conclude from this test that the three rats with transplanted eyes had some vision.

## SUMMARY

1. The extirpated eyeballs of the Norwegian spotted rat can be successfully replanted and in a small percentage of cases varying degrees of recovery obtained.

a. In some cases we secured only the clearing up of the cornea and the return of the corneal reflex, the lens remaining opaque and the pupil irregular.

b. After transplantation a vascularization of the cornea appears. This vascularization always disappears in about four weeks.

c. In some cases motility of the eyball returns.

d. In three cases we observed the *return* of the reaction of the pupil to light.

2. Three animals with transplanted eyes showed negative phototaxis and positive Waugh test indicating some return of vision.

The authors gratefully acknowledge their indebtendess to Doctors Carlson and Ivy for their valuable suggestions and coöperation and also for many other kindnesses.

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# CHEMICAL STUDIES OF THE OVIDUCT OF THE HEN

G. DAVIS BUCKNER, J. HOLMES MARTIN AND A. M. PETER

*From the Kentucky Agricultural Experiment Station, Lexington*

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In studying the calcium metabolism of the laying hen with special reference to the physiology of egg production, it became desirable to learn something of the chemical processes taking place in the oviduct of the laying hen, and in what way they differ from those of the resting period.

The histology of the oviduct of the hen has been studied by Surface (1), with bibliography, and Pearl and Curtis (2) have investigated, in a measure, the physiology of reproduction of the hen with special reference to the oviduct.

It is a matter of general knowledge that the oviduct of the fully matured pullet that has never laid is small when compared to that of the actively laying individual, and that a partial atrophy of this organ accompanies the cessation of egg-laying. Kaupp (3) estimates that the oviduct of a Plymouth Rock pullet whose reproductive organs have never become active, is about 11 cm. long, but that when fully developed and in the actively laying condition it is from 45 to 50 cm. long.

The oviduct usually is divided into 5 parts, the funnel, the albumen-secreting part, the isthmus (membrane secreting part), the uterus (shell secreting part) and the vagina (cuticle secreting part). These sections can be separated. As each section plays a distinct rôle in the formation of the egg, it seems to us that the composition of each must be different and that a knowledge of these differences would throw more light on the problem under investigation in this laboratory. We therefore decided to make comparative analyses of the albumen-secreting section, the isthmus and the uterus from hens whose egg-laying capacities were known, as well as their laying condition. With this end in view we selected 8 two-year-old, single-comb, White Leghorn hens that had come from the same parent stock and had been hatched the same day. Each of these hens laid between 150 and 173 eggs the first year. They had existed under identical conditions since hatching. Also, a year-old hen of the same breed, that had never laid, was selected. These nine hens were killed by dislocating their necks, and their oviducts carefully dissected out. Each oviduct, after it had been cut free from the inferior and superior ligaments and adhering tissues, was washed out carefully with distilled

water and a glass rod about 75 mm. in diameter passed through it. In this manner the different segments could be measured and separated. The three desired sections and the washings were placed in separate platinum dishes and dried in an electric oven at 100 °C. for 24 hours, and weighed. These dried materials were carefully burned at a low heat, digested with HCl, filtered and reburned. This was necessary because the carbon could not be burned out of the untreated residues at a low heat, indicating an excess of phosphorus over the bases present. The residues were treated with HCl, the solutions filtered, and the filtrates and washings made to

TABLE 1

*Analyses of the albumen-secreting part of the oviduct*

| HEN NUMBER | CONDITION OF HEN | LENGTH OF PART | DRY WEIGHT | TOTAL CaO | TOTAL $P_2O_5$ | PER CENT CaO IN DRIED MATERIAL | PER CENT $P_2O_5$ IN DRIED MATERIAL |
|---|---|---|---|---|---|---|---|
| | | *cm.* | *grams* | *grams* | *gram* | *per cent* | *per cent* |
| 154 | Heavy laying | 38.5 | 4.72 | 0.0042 | 0.0887 | 0.09 | 1.88 |
| 158 | | 37.5 | 5.55 | 0.0050 | 0.0949 | 0.09 | 1.71 |
| 164 | | 41.0 | 5.63 | 0.0056 | 0.1109 | 0.10 | 1.97 |
| Total | | 117.0 | 15.90 | 0.0148 | 0.2945 | 0.28 | 5.56 |
| Average | | 39.0 | 5.30 | 0.0049 | 0.0982 | 0.09 | 1.85 |
| 156 | Nearly ceased laying | 35.5 | 5.20 | 0.0057 | 0.2179 | 0.11 | 4.19 |
| 166 | | 40.0 | 3.71 | 0.0037 | 0.0894 | 0.10 | 2.41 |
| Total | | 75.5 | 8.91 | 0.0094 | 0.3073 | 0.21 | 6.60 |
| Average | | 37.8 | 4.46 | 0.0047 | 0.1537 | 0.11 | 3.30 |
| 160 | Resting period | 25.5 | 0.97 | 0.0004 | 0.0277 | 0.04 | 2.86 |
| 168 | | 26.5 | 0.86 | 0.0012 | 0.0193 | 0.14 | 2.24 |
| 170 | | 26.5 | 0.77 | lost | 0.0196 | lost | 2.55 |
| Total | | 78.5 | 2.60 | 0.0016 | 0.0666 | 0.18 | 7.65 |
| Average | | 26.2 | 0.87 | 0.0008 | 0.0222 | 0.09 | 2.55 |
| 198 | Had never laid | 13.5 | 0.08 | 0.0001 | | 0.07 | |

100 cc. with distilled water. Calcium and phosphorus were determined in separate aliquots, the former by the method of McCrudden (4), and the latter by the volumetric method of the Association of Official Agricultural Chemists (5). The percentages of the oxides of these elements were calculated in the dried materials.

The 9 hens supplying the material for this experiment were divided into four groups.

1. Three hens, numbers 154, 158 and 164, were in good laying condition; in fact, when killed, the first two had fully developed eggs in the uterus, while in hen 164 the uterus contained an egg with the shell par-

tially deposited. This egg was fully distended, yet the thin deposit of shell permitted perfect flexibility.

2. Two hens, numbers 156 and 166, had been laying heavily but had practically ceased to lay and were molting.

3. Three hens, numbers 160, 168 and 170, were in the resting state, not having laid an egg for at least a month, and were in an advanced stage of moult.

4. One hen, number 198, though one-year-old, had never laid an egg.

All hens except no. 198 were killed at the same time and their condition noted.

TABLE 2

*Analyses of isthmus of oviduct*

| HEN NUMBER | CONDITION OF HEN | LENGTH OF PART | DRY WEIGHT | TOTAL CaO | TOTAL $P_2O_5$ | PER CENT CaO IN DRIED MATERIAL | PER CENT $P_2O_5$ IN DRIED MATERIAL |
|---|---|---|---|---|---|---|---|
| | | *cm.* | *grams* | *gram* | *gram* | *per cent* | *per cent* |
| 154 | Heavy laying | 10.0 | 0.76 | 0.0034 | 0.0185 | 0.45 | 2.44 |
| 158 | | 11.0 | 0.83 | 0.0039 | 0.0205 | 0.47 | 2.47 |
| 164 | | 11.0 | 0.91 | 0.0036 | 0.0199 | 0.40 | 2.19 |
| Total | | 32.0 | 2.50 | 0.0109 | 0.0589 | 1.32 | 7.10 |
| Average | | 11.0 | 0.83 | 0.0036 | 0.0196 | 0.44 | 2.37 |
| 156 | Nearly ceased | 9.0 | 0.71 | 0.0034 | 0.0193 | 0.48 | 2.72 |
| 166 | laying | 9.0 | 0.49 | 0.0023 | 0.0148 | 0.47 | 3.03 |
| Total | | 18.0 | 1.20 | 0.0057 | 0.0341 | 0.95 | 5.75 |
| Average | | 9.0 | 0.60 | 0.0029 | 0.0171 | 0.48 | 2.88 |
| 160 | Resting period | 9.0 | 0.32 | 0.0004 | 0.0088 | 0.12 | 2.74 |
| 168 | | 9.0 | 0.33 | 0.0012 | 0.0110 | 0.36 | 3.33 |
| 170 | | 9.0 | 0.30 | 0.0006 | 0.0095 | 0.19 | 3.18 |
| Total | | 27.0 | 0.95 | 0.0022 | 0.0293 | 0.67 | 9.25 |
| Average | | 9.0 | 0.32 | 0.0007 | 0.0098 | 0.22 | 3.08 |
| 198 | Had never laid | 7.0 | 0.08 | 0.0004 | | 0.50 | |

When dilute HCl was applied to any part of the interior of the wet oviduct, no evolution of $CO_2$ was observed, nor was there any from the carefully carbonized materials, including the dried washings. This proves the absence of an appreciable amount of carbonate or bicarbonate.

Table 1, giving the analyses of the albumen-secreting portion of the oviduct, shows that this part becomes shorter as the resting period is approached, but even then it is approximately twice as long as in the one-year-old hen that never laid. There is a decided lessening in the weight as shown by the average of 5.3 grams for the active laying hens and 0.87 gram for those in the resting state. These weights are large when com-

pared to the very small weight of 0.08 gram in the non-laying hen. Evidently, this part of the oviduct becomes both thinner and shorter, as the resting period is approached, but the extreme thinness observed in the non-laying hen is not reached, even in the resting period.

The total weight of calcium decreases with the total weight of the organ, while the percentage in the dry matter remains nearly constant.

The total weight of phosphorus is greater in the second group than in the first, but less in the third group than in the first, whereas the percentage in the dry matter is greater in the second group and less in the first than in the third.

TABLE 3

*Analyses of the uterus*

| HEN NUMBER | CONDITION OF HEN | LENGTH OF PART | DRY WEIGHT | TOTAL CaO | TOTAL $P_2O_5$ | PER CENT CaO IN DRIED MATERIAL | PER CENT $P_2O_5$ IN DRIED MATERIAL |
|---|---|---|---|---|---|---|---|
| | | *cm.* | *grams* | *gram* | *gram* | *per cent* | *per cent* |
| 154 | Heavy laying | 10.0 | 1.92 | 0.0040 | 0.0483 | 0.21 | 2.52 |
| 158 | | 11.0 | 2.43 | 0.0051 | 0.0547 | 0.21 | 2.25 |
| 164 | | 11.0 | 2.24 | 0.0067 | 0.0540 | 0.30 | 2.41 |
| Total | | 32.0 | 6.59 | 0.0158 | 0.1570 | 0.72 | 7.18 |
| Average | | 11.0 | 2.20 | 0.0053 | 0.0523 | 0.24 | 2.39 |
| 156 | Nearly ceased laying | 10.0 | 2.29 | 0.0071 | 0.0600 | 0.31 | 2.62 |
| 166 | | 9.0 | 1.68 | 0.0035 | 0.0435 | 0.21 | 2.59 |
| Total | | 19.0 | 3.97 | 0.0106 | 0.1035 | 0.52 | 5.21 |
| Average | | 10.0 | 1.99 | 0.0053 | 0.0517 | 0.26 | 2.61 |
| 160 | Resting period | 9.0 | 0.75 | 0.0024 | 0.0180 | 0.32 | 2.40 |
| 168 | | 9.0 | 0.76 | 0.0014 | 0.0194 | 0.18 | 2.56 |
| 170 | | 9.0 | 0.78 | 0.0004 | 0.0176 | 0.05 | 2.26 |
| Total | | 27.0 | 2.29 | 0.0042 | 0.0550 | 0.55 | 7.22 |
| Average | | 9.0 | 0.76 | 0.0014 | 0.0183 | 0.18 | 3.41 |
| 198 | Had never laid | 9.0 | 0.46 | 0.0005 | | 0.11 | |

In table 2 we note only a slight shortening of the isthmus, but the weight decreases to approximately 30 per cent in the resting state. The weight of the isthmus in the non-laying hen is very small. The weights of CaO and $P_2O_5$ in this part decrease as the resting period is approached. The percentage of CaO in the isthmus of a laying hen and in one that is ending her egg-laying season is approximately the same as it is in a non-laying hen but is approximately 50 per cent less in one during the resting period. The percentage of $P_2O_5$ in the isthmus increases somewhat as the resting period is approached.

In table 3 we see only a slight shortening in the uterus, yet a marked lessening in the thickness is shown by the decrease from 2.23 grams in the heavy laying hen to 0.76 gram in the hen in the resting period. The quantities of CaO and $P_2O_5$ decrease as the resting period approaches. The percentage of CaO decreases and the percentage of $P_2O_5$ increases from the heavily laying uterus to the resting state.

In table 4 we note a decrease in the dry weights of the washings of the oviduct and the quantities of CaO and $P_2O_5$ become smaller as the resting

TABLE 4

*Analyses of the washings from the inside of the oviduct*

| HEN NUMBER | CONDITION OF HEN | DRY WEIGHT | TOTAL CaO | TOTAL $P_2O_5$ | PER CENT CaO IN DRIED MATERIAL | PER CENT $P_2O_5$ IN DRIED MATERIAL |
|---|---|---|---|---|---|---|
| | | *gram* | *gram* | *gram* | *per cent* | *per cent* |
| 154 | Heavy laying | 0.10 | 0.0012 | 0.0048 | 1.22 | 4.75 |
| 158 | | 0.13 | 0.0039 | 0.0065 | 3.00 | 4.97 |
| 164 | | 0.08 | 0.0059 | 0.0063 | 7.37 | 7.87 |
| Total | | 0.31 | 0.0110 | 0.0176 | 11.59 | 17.59 |
| Average | | 0.10 | 0.0037 | 0.0059 | 3.86 | 5.86 |
| 156 | Nearly ceased | 0.07 | 0.0006 | 0.0029 | 0.82 | 4.11 |
| 166 | laying | 0.18* | 0.0021 | 0.0072 | 1.20 | 3.99 |
| Total | | 0.25 | 0.0027 | 0.0101 | 2.02 | 8.10 |
| Average | | 0.13 | 0.0014 | 0.0051 | 1.01 | 4.05 |
| 160 | Resting period | 0.07 | 0.0014 | 0.0023 | 2.00 | 3.29 |
| 168 | | 0.03 | 0 | 0.0022 | 0 | 7.39 |
| 170 | | 0.02 | 0.0002 | 0.0019 | 1.20 | 9.67 |
| Total | | 0.12 | 0.0016 | 0.0064 | 3.20 | 20.35 |
| Average | | 0.04 | 0.0005 | 0.0021 | 1.07 | 6.78 |
| 198 | Had never laid | 0.14 | 0.0002 | | 0.14 | |

* There was a small piece of white horny material about the size of a pin head in the washings from this uterus.

period nears. The percentage of CaO is smaller and the percentage of $P_2O_5$ is larger toward the resting state.

It is most interesting to note the relatively small amount of calcium in the oviduct when we realize that approximately 5.0 grams of $CaCO_3$ is deposited in the form of egg-shell during the 15 or 16 hours the egg remains in the uterus. The fact that the percentage of CaO in the oviduct is lessening as the resting period approaches and the percentage of $P_2O_5$ is increasing indicates that the calcium deposited as eggshell is not associated with the phosphorus of the oviduct but comes to the uterus in the blood stream in another form.

Besides the general atrophy of the oviduct during the resting period, the lessening in the engorgement of the blood vessels of the organ is very noticeable. The atrophied oviduct does not decrease to the size of the oviduct of a non-laying hen.

It has been always noticed in this laboratory that eggs found in the uterus of autopsied hens, whether without shell or with an incomplete shell were turgid, that is, filled with material.

In conclusion we hope to throw further light on the subject of the chemistry of the formation of the egg in the uterus of hens, by a continuation of this investigation.

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# THE METABOLISM OF AMMONIUM SALTS AND OF UREA IN MAN

EDWARD F. ADOLPH

*From the University of Pittsburgh*

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These experiments were designed to find how much of an ingested quantity of urea or of an ammonium salt is eliminated, and how rapidly the elimination proceeds. No previous experiments upon this subject have been reported upon man. Several factors were found to influence the elimination, the chief one peculiar to these compounds being the acid-base balance of the body. The metabolism of acid-producing and alkali-producing ammonium salts was then compared. The acid-base influence upon the elimination of nitrogen appears to explain the discrepancies found among the extensive experiments made by numerous investigators upon the metabolism of ammonia-nitrogen in various mammals.

The metabolism study was made upon a single human subject (E. F. A.), weighing 70 kilograms. The various compounds were ingested within 2.5 hours after a light breakfast. No food or water was taken throughout the day, a period of 8 to 12 hours. Voluntary urine samples were collected at approximately one-hour intervals during this time. In the urine samples chlorides were determined by Harvey's (1) titration method; urea by the clinical method of Marshall (2); ammonium salts by the formalin titration of Malfatti (3); acid and alkali by titration to methyl orange, phenolphthalein, or alizarin; and hydrogen ion concentration by colorimetric comparison with the phosphate standards of Sörensen (4). Samples of alveolar air were collected and analyzed for $CO_2$ by the method of Haldane and Priestley (5).

It is a pleasure to acknowledge my indebtedness to Dr. J. S. Haldane in Oxford and to the U. S. Bureau of Mines in Pittsburgh for providing the laboratory facilities used in performing these experiments.

THE ELIMINATION OF INGESTED SUBSTANCES. *Normal elimination.* Data upon the normal elimination of endogenous substances in the urine were gathered upon 10 control days. The normal hourly elimination of water and of chloride (E. F. A.) have been indicated graphically in another paper (6). It was there shown that the urinary elimination during any hour of the day may be predicted with some accuracy from the rate of excretion for one or two morning hours.

The normal excretion of endogenous urea upon 7 normal days is indicated in the accompanying figure 1. An average curve is also shown. It will be seen that the curve which represents urea elimination by man after a light breakfast is of the shape found by McEllroy and Pollock (7) for the urea elimination by dogs fed a meal rich in protein. Since the curve has the same shape for a nitrogenous output of any size, it is not even necessary, in predicting hourly elimination, that the meal shall be a uniform one.

*Elimination of ingested salts.* When a salt, e.g., sodium chloride, was ingested, its elimination was measured quantitatively by correcting for

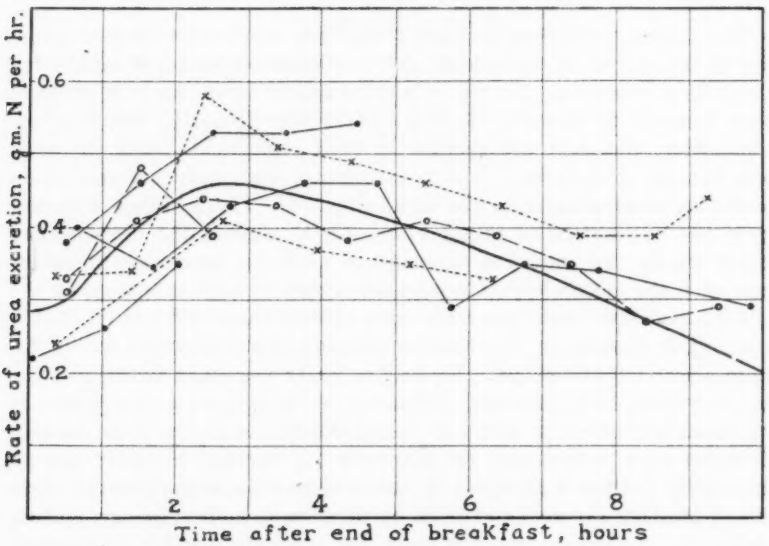


Fig. 1. The rate of urea excretion upon 7 normal days. Subject, E. F. A. The shape of the smoothed average curve was used in calculating the expected nitrogen elimination during the experiments.

the normal elimination of that salt, knowing the rate of excretion which prevailed before the ingestion was made. It was found that some of the salt, but not all of it, was eliminated before excretion returned to its predicted normal rate. Complete elimination of chloride within 24 hours occurred only in one case, namely, when ingested in the form of ammonium chloride. Two factors were significant in controlling the amount of chloride eliminated. First, an increase in the amount of salt ingested at one time led to greater percentage elimination. Second, an increased amount of water in the body slightly increased the elimination (6), and a decreased amount of body water decreased the elimination (8).

At normal levels of water excretion, sodium chloride was eliminated, during the period of abnormally high rate of chloride excretion, to the extent of 30 to 75 per cent, when ingested in doses of 15 grams or more (average 59 per cent); 11 to 21 per cent when taken in smaller doses (average 14 per cent).

TABLE 1

*Nitrogen elimination and acid-base equilibrium after the ingestion of ammonium salts and urea. Subject, E. F. A.*

| EXPERIMENT NUMBER | INGESTION | | | | ELIMINATION | | | ALVEOLAR $CO_2$ TENSION | | pH OF URINE | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Substance | Grams of nitrogen | Cubic centimeters of water | Hours after breakfast | Hours duration of excessive urea excretion | Grams excessive urea-nitrogen excreted during this time | Per cent of ingested nitrogen excreted as excessive urea | Maximum increase, in mm. of mercury | Time of increase, in hours after the ingestion | Maximum pH | Time of increase, in hours after the ingestion |
| 7 | $NH_4Cl$ | 3.9 | 200 | 1.8 | – | – | – | −6.1 | 2 to 52 | 6.0 | – |
| 53 | $NH_4Cl$ | 3.1 | 100 | 2.2 | Vomited after 50 minutes. | | | | | | |
| 8 | $NH_4HCO_3$ | 2.9 | 150 | 1.6 | – | – | – | −1 0 | 2.5 to 5.5 | 6 0 | – |
| 37 | $NH_4HCO_3$ | 6 0 | 100 | 1.5 | > 6 | 0.6 | 10 | – | – | 8.0 | 2.5 to 3.5 |
| 41 | $(NH_4)_3$ citrate | 3.5 | 100 | 2.0 | 6 | 0 2 | 5 | – | – | 7.6 | 2.0 |
| 38 | Urea | 2.3 | 25 | 1.5 | >5 | 0.4 | 16 | – | – | 8 0 | 2.5 to 3.5 |
| 40 | Urea | 2.1 | 250 | 2.5 | >4 | 0 7 | 31 | – | – | 7 8 | 2.5 to 4.6 |
| 10 | Urea | 7.0 | 100 | 1.8 | – | – | – | +2.4 | 2.3 | 6.8 | 3.8 |
| 14 | Urea | 14.0 | 120 | 1.0 | – | – | – | −3 5 | 3.1 to 6.4 | 6.0 | – |
| 16 | Urea | 14.0 | 120 | 1.2 | >14 | 10.5 | 75 | +2.0 | 0.6 to 6.1 | 7.3 | 2.1 |
| 29 | Urea | 14.0 | 150 | 1.4 | >13 | 7.5 | 53 | – | – | – | – |
| 18 | Urea | 14.0 | 120 | 1.1 | >23 | 8.2 | 59 | +1.1 | 1.3 | 6 6 | 2.1 |
| 19 | Urea | 21.0 | 180 | 0 7 | 23 | 13.1 | 62 | +0.9 | 1.6 | 6.4 | 0.9 to 6.0 |
| 23 | Urea + 15 grams NaCl | 14.0 | 200 | 1.5 | 15 | 10.7 | 76 | 0 | – | 6.0 | – |
| 30 | Urea + 15 grams NaCl | 21.0 | 255 | 1.1 | 23 | 18.6 | 88 | – | – | 6.0 | – |
| 32 | Urea + 15 grams NaCl | 11.7 | 0 | 0 | >16 | 10.0 | 85 | – | – | 7.4 | 2.3 |

*Elimination of ingested urea and ammonium salts.* All the experiments in which nitrogenous compounds were ingested are given in table 1. These data show that the same two factors found for sodium chloride influenced the amount of elimination of urea and ammonium salts. First, when only small quantities of the nitrogenous compounds were ingested (up to 0.10 gram of nitrogen per kilo) only 5 to 16 per cent of the nitrogen was eliminated (average 11 per cent). When larger quantities were taken (0.18

to 0.30 gram of nitrogen per kilo) 53 to 88 per cent was eliminated (average 62 per cent). Second, excessive water ingestion increased the elimination (compare experiments 38 and 40); thirst reduced the elimination (see experiment 19).

There was no difference in the manner of elimination whether the nitrogen was ingested as ammonium bicarbonate, ammonium citrate or urea. In all the above cases the excessive nitrogenous output was in the form of urea, while the output of ammonium salts was unchanged. When, however, the ingested nitrogen was ammonium chloride, the ammonium excretion was increased with the urea.

The retention of nitrogen after urea ingestion is evidently due to the temporary storage of urea as such in the tissues, exactly as chloride is retained as such after its ingestion; the amount of retention is the same in both cases. No one would suppose that the excessive amount of either substance did more than dilute the preëxisting body substances, just as ingested Locke's solution temporarily does (8).

The conclusion that the retention of urea is similar to that of inorganic salts is adverse to the theory of Grafe and Schläpfer (9) that ingested ammonia can effectively help in forming tissue substance in the mammalian organism. It supports the conclusions of Abderhalden and Hirsch (10) and Cathcart (11) that "if the body can synthesize amino acids from urea or ammonium salts it does not readily do so."

THE INFLUENCE OF THE ACID-BASE EQUILIBRIUM. *Alkalosis.* Several of our experiments show that substances which produce alkalosis in the body lead to incomplete elimination of ingested nitrogen. Thus, when an experimental alkalosis was produced by ingesting sodium bicarbonate, the rate of excretion of endogenous urea was significantly reduced. Such a reduction did not occur when sodium chloride or water were taken instead of the bicarbonate. A similar reduction of urea excretion has been reported by Nagayama (12) when sodium bicarbonate was administered to rabbits.

In several of the experiments in which urea, ammonium bicarbonate or ammonium citrate was ingested, a slight alkalosis resulted (13). The degree of alkalosis caused by these ingesta, however, is too slight to lead to a measurable nitrogen retention; for the average retention of urea, as noted above, was practically the same as the retention of chloride when sodium chloride was ingested (38 and 41 per cent respectively).

The retention of nitrogen under the same circumstances has, however, been repeatedly observed upon animals other than man. Thus, Taylor and Ringer (14) found that nitrogen was partially or sometimes almost completely retained by dogs which were fed either urea or ammonium carbonate. It may also be noted that neither in their experiments nor in ours did any of these substances lead to an absolute or relative increase in the ammonium excretion.

*Acidosis.* Acidosis was produced experimentally in the human body by ingesting ammonium chloride in one experiment. This method and its results have been described by J. B. S. Haldane (15), (16). The acidosis lasted for a considerable period, lowering the alveolar $CO_2$ tension during 48 hours. Due to the long duration of the acid condition, it was impossible to measure the nitrogenous output with any accuracy by the method of study used. But the rapid excretion of chloride which occurred suggested that all the ingested substance was eliminated rapidly (within 12 hours), and that urea as well as chloride was eliminated over and above the ingested amount. Unfortunately further experiments of this kind were prevented by the inability of the subject to retain the salt in the gastrointestinal tract (experiment 53).

Another experiment was performed in which acidosis was produced by breathing an atmosphere containing carbon dioxide. The subject (E. F. A.), having ingested nothing for 16 hours, sat quietly in a "man house" for 2 hours, breathing 5.2 per cent of $CO_2$. The alveolar $CO_2$ tension rose at first from 39.5 mm. of mercury to 48.0 mm., and later remained steady at 45.5 mm. The breathing was doubled in frequency and trebled in depth. During the 2-hour period the urea excretion remained constant, while the ammonia excretion gradually rose. As soon as the experiment ended, however, the ammonia elimination rose still more rapidly, and the rate of urea excretion increased 50 per cent. The water excretion increased only during the exposure to $CO_2$, due to the high excretion of acid (6), and dropped below normal immediately afterwards. The chloride output was parallel to the water output.

These two acidosis experiments, hardly significant by themselves, are adequately supplemented by the observations of other investigators. Haldane (15) measured the ammonia excreted after he had ingested ammonium chloride, and found that the urinary ammonia increased so far above normal that it accounted for half of the nitrogen ingested. The data of Salkowski (17) on rabbits and dogs, and of Underhill (18) on dogs, show a similar high ammonia elimination when the animals were fed various *inorganic* salts of ammonia. In further experiments Underhill and Goldschmidt (19) found that while nitrogen might be retained by the dogs after they had ingested organic ammonium salts, no nitrogen was retained and more nitrogen than that ingested was excreted when they had taken inorganic ammonium salts. It is only necessary to realize that the inorganic salts all produce acidosis, to see that this factor is the common one in all cases of high nitrogen elimination.

McCollum and Hoagland (20) showed that any acid salts fed to pigs not only led to a high excretion of ammonia, but that this ammonia was in excess of the nitrogen normally eliminated. Keeton (21) showed that acid given intravenously to dogs increased the excretion of both ammonia and total nitrogen. These results are only a few of those recorded in the lit-

erature which support the view that acidosis leads to excessive elimination of ammonia and urea nitrogen.

MECHANISM OF NITROGEN MOBILIZATION IN ACIDOSIS. There are several possible ways in which the excretion of nitrogen may be increased by acid and decreased by alkali. Practically all the views concerning the nature of the increase which have been put forward so far, assume that the increase is for the purpose of furnishing ammonia to neutralize the acid.

One of these views is that preformed ammonia is drawn from the body's protoplasmic mass, urea nitrogen presumably being unable to neutralize the excess of acid (20). Now, although Wakeman and Dakin (22) demonstrated that in perfusion experiments urea cannot be converted into ammonia in the dog's liver, yet Nash and Benedict (23) and Ambard (24) have shown that ammonia can be formed rapidly in the kidneys, perhaps from urea. Folin and Denis (25) and Henriques (26) found in some carnivorous animals a higher ammonia content in the portal blood than in the systemic blood, showing that ammonia is constantly being formed in the intestine, presumably by bacteria. It is apparent that, when needed for neutralizing acid, plenty of ammonia is available, and therefore that the assumption that protoplasmic ammonia must be drawn upon is invalid.

A second view is that acidity speeds up the catabolic processes of the body's protoplasm, leading to increased elimination of all waste products (18). Upon this view the acids, which favor the activity of autolytic enzymes, counterbalance the basic substances, which favor anabolism, so that there is an excess of catabolism over anabolism. This recalls the definition of "growth" given by Verworn (27) as the excess of anabolism over catabolism. But Salkowski (17) proved conclusively that catabolism is not stimulated by ammonium chloride ingestion, for he found that the sulfur excretion was not augmented while the nitrogen excretion was.

A hypothesis which seems to fulfil the requirements of our present knowledge is, that during the excretion of acid both urea and ammonia pass through the kidneys more readily than normally. This view implies that there is no change in the rate of production of these substances by the tissues at these times, but only a more rapid elimination of them. No measurable change in the blood concentration of urea is necessarily produced, since the whole body is contributing the excess. From all the evidence brought together it is clear that the elimination of only ammonia and urea is increased by the presence of excessive acid in the body, and not that of water and chloride. Such a specificity is characteristic of the selective activity of the kidneys.

If, as suggested, the acidity of the blood influences the activities of the kidneys, then the excretory constants which have been worked out for the excretion of these two substances, urea and ammonia, must be modified by the changes in acid-base equilibrium.

## CONCLUSIONS

1. Most dissolved substances taken into the human body are not completely eliminated over short periods of time. Curves indicating their rate of elimination at various times after ingestion have been constructed. Under standardized conditions the retention over 12-hour periods is equally great for sodium chloride, urea, ammonium bicarbonate and ammonium citrate.

2. The form in which ingested ammonia-nitrogen is eliminated does not depend upon whether it was ingested as ammonia or as urea, but upon the acid-base equilibrium in the body.

3. The amount of an ingested ammonium salt or urea which is retained depends also upon the acid-base conditions in the body.

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# THE EFFECT OF COPULATION, PREGNANCY, PSEUDOPREGNANCY AND LACTATION ON THE VOLUNTARY ACTIVITY AND FOOD CONSUMPTION OF THE ALBINO RAT[1]

JAMES ROLLIN SLONAKER

*From the Department of Physiology at Stanford University, California*

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In a recent paper (1) we discussed the effect of pubescence, oestruation and the menopause on the voluntary activity of the albino rat and showed that during sexual life the female exhibited marked rhythmical fluctuations in activity which corresponded to and were identical with the oestrual cycles, the peaks of activity coinciding with oestrum. Though much individual variation was found these spurts of activity occurred with great regularity approximately every fourth day in young females. In older females the interval between peaks of activity was lengthened averaging from five to five and one-half days. As the animals approached the menopause (755 days) the oestrual rhythm was much disturbed and showed periods of dioestrum as great as 45 days. Though small spurts of activity in animals prior to the age of sexual maturity occurred there was no regularity in their distribution; that is, no rhythm was manifested. After the menopause the curve of activity represented that of senility. We also emphasized the fact that a rhythm in activity corresponding to the oestrual rhythm exhibited by females during their sexual life was not shown by males nor by ovariectomized females, but was shown by hysterectomized females. Our results confirmed the investigations of others in that the oestrual rhythm was due to the functioning ovary.

In this paper we will present our results on the effect of mating, gestation, pseudopregnancy and lactation on voluntary activity and the food consumption, to show how the normal oestrual rhythm is influenced by lack of ovulation.

In a recent paper Wang (2) has shown that the oestrual rhythm is suppressed during gestation and lactation. He weaned the young at the age of twenty days and found that the normal rhythm was not reëstablished until ten days later. While we have had many cases which substantiate his results we also found great individual variation and many

[1] This research has been conducted with the aid of the Department of Physiology and the Research Fund of Stanford University, and The Committee for Research on Sex Problems of the National Research Council.

cases of earlier oestrus. Since he used but four females, his observations were too limited to demonstrate many modifications which we have found. We have had cases where oestruation occurred during gestation and during lactation as early as the day of birth of the litter and at various times afterward.

Ross-Johnson and Hewer made eight mating tests and found that the interval between the birth of the first litter and the birth of the second litter ranged between twenty-three and thirty-four days. Since the second gestation required approximately twenty-two days of this time, ovulation and fertilization must have occurred from the first to the twelfth day of lactation. They further verify these results by examination of ovaries of mothers killed the twenty-first day of lactation. From the presence of young corpora lutea in these ovaries they concluded that ovulation does occur during lactation in the rat.

By means of vital stains and vaginal smears Long and Evans (4) conclude that ovulation does not occur during lactation and that "instances of pregnancy occurring early in lactation are due to fruitful copulation on the day of littering."

Cases of ovulation having occurred during lactation have been reported by King (5) for the rat and by Daniel (6) and Kirkham (7) for mice. Daniel also states that lactation during gestation tends to lengthen the period of gestation.

Loeb and Kuramitsu (8) in their investigation concerning the influence of lactation on the sexual cycle in the rat and guinea pig compared the ovaries of nursing mothers with those of mothers whose young had been removed at birth. They found the ovaries of non-nursing mothers larger and containing more corpora lutea of various ages than those of nursing mothers. This difference was noticed as early as seven days and as late as four weeks after labor. Five weeks after labor follicles were found in both but were generally larger in the non-nursing than in the nursing mothers. From these observations they conclude that ovulation is suspended during the period of lactation.

The results of many other investigations relative to the effect of gestation and lactation on ovulation and the oestrual cycles could be given. The above, however, will suffice to show that there is a marked divergence in the results. This difference may have been due to a lack of sufficient number of observations, to an insufficient frequency of tests on the same individuals, to handling or other environmental changes, or to many other causes. In our activity experiments, where we had the isolated animal under continuous observation in practically a uniform environment we have found that often slight changes in conditions, such as cleaning the cages, resulted in a disturbance of voluntary activity lasting from one to three days. Our results also show individual cases which substantiate

all the results quoted above. In general, however, we find that gestation and lactation tend to suppress ovulation. In some cases it may be completely suppressed during gestation and at least the first twenty days of lactation while in other cases it is only partially suppressed.

*Materials.* In this experiment twenty-five female rats, all in the prime of life, were used. Each rat was isolated in a separate revolving cage (previously described (9)), which was its home during the experiment. The stationary nest box, which was just large enough to permit the rat to sleep comfortably, carried the food and water boxes and was supported to the axle of the cage. The advantage of this equipment is that practically all the voluntary activity was manifested in turning the revolving cage. The number of revolutions were recorded automatically on Veeder counters. The animals were fed and watered daily at 7 o'clock p.m., at which time the counters were read and reset to zero. The counters were also read at approximately 8 o'clock each morning. The two readings thus gave us not only the total run for the twenty-four hours, but also for each day and night. The results of these readings confirmed our former statement that the albino rat, like the wild rat, is practically a nocturnal animal, exhibiting little activity during the day time.

During the preliminary stages of the experiment, the first three months, the rats were fed a commercial chick food known as "Suregrow" made by the Sperry Flour Co., which, according to the manufacturers, contained the following ingredients: bran, shorts, middlings, flour, corn grits, corn meal, bone meal, meat scraps, cocoanut meal, oatmeal, charcoal and dry buttermilk. Their analysis gave minimum crude protein, 17 per cent; minimum crude fat, 4 per cent; maximum crude fiber, 6 per cent; and maximum crude ash, 7 per cent. Though the rats did well on this diet we did not feel sure that its composition would remain absolutely the same all the time. We therefore changed, for the remaining five months of the experiment, to a modified McCollum diet. This consisted of the following proportions by weight: ground whole wheat, 3375; whole milk powder, 500; commercial casein, 750; sodium chloride, 50; calcium carbonate, 75; sifted ground alfalfa, 150; milk fat, 250. The dry materials were first thoroughly mixed then the fat added and stirred in until the lumps were reduced to a small size. The mass was then put through a grinder which reduced it to a uniform consistency. Further stirring after grinding distributed all the ingredients uniformly throughout the whole mass. Sufficient food to last two or three days was prepared at a mixing.

The water and food boxes were made of sheet copper. The water box was 50 × 50 × 50 mm. and had a capacity of 125 cc. This assured an abundance of water at all times. The food box was 50 × 50 and 80 mm. deep and had a capacity of 200 cc. A maximum of about 80 grams of the diet could be put into the food box at one time. In case of large litters

when a greater amount of food would be consumed during the twenty-four hours during the later days of nursing, an additional quantity was given at the morning reading.

It was soon found that there was a tendency on the part of some of the animals to waste the food by pawing it out of the boxes. Since this made it impossible to determine the amount of daily consumption, removable hoods, which slipped securely into the food boxes, were made. Figure 1*A* shows the removable hood and the food box, *B*, in relative position. This arrangement permitted the rat to reach its food only through the opening, *O*, which was just sufficiently large to allow easy entrance of the head but prevented free use of the fore feet. This arrangement practically prevented all waste and made possible accurate determination of the daily food consumption. More food was given to each rat than it could eat during the twenty-four hours. The amount consumed therefore was readily ascertained by deducting the weight of the box and uneaten food from its weight the previous day.

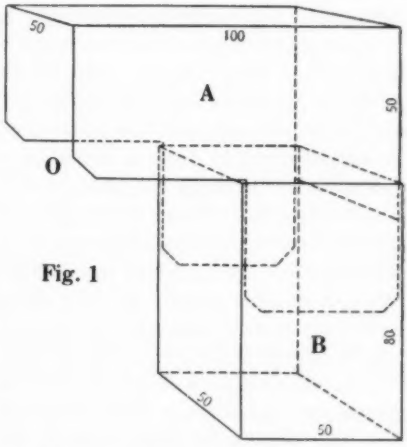


Fig. 1

Fig. 1. Assembled food box, *B*, and removable cover, *A*, showing entrance to food at *O*. Dimensions are in millimeters.

The curves of activity and food consumption for each rat were plotted daily. In this way we could determine just when oestrus would occur and make our matings. In making matings the female was removed from the revolving cage and placed with a breeding male in a stationary cage for from ten minutes to one hour. All such matings occurred in the forenoon and the exact hour was recorded. Observations were made to see if copulation resulted. In many cases vaginal smears were also made to determine if insemination had occurred. In all cases the later curve of activity gave the same information. It was soon seen that many copulating acts did not result in fertilization. Many deliveries happened in the day time and the time noted. Knowing the date and the time of copulation and delivery we were able to compute the approximate length of the gestation period and were thus able to see what effect the size of the litter had on the length of the gestation period. It has been shown by various writers that the time of ovulation relative to heat varies. Owing to this we were

unable to determine the time of fertilization and the exact gestation period.

The number, sex and weight of the young were ascertained as soon after birth as possible. Their weights were also taken at varying ages afterward. Weaning usually occurred at the age of twenty days. Some, however, were allowed to nurse for thirty or more days to further show the effect of lactation on oestrus. Results after the young had reached the age of twenty-five to thirty days were not very satisfactory, for at this age the young are likely to leave the nest and get into the revolving cage thus interfering with the voluntary activity of the mother.

The experiment was continued approximately eight months during which time fifty-six successful matings and deliveries occurred. Some of these were not used owing to incomplete observations.

*Average activity and food consumption.* The average number of revolutions voluntarily turned daily by the non-pregnant female rat in the prime of life varied between 8000 and 10,000 revolutions and the average daily food consumption was approximately 18 grams. Extreme variations in activity ranged from a few thousand to 44,640 revolutions. During gestation the average voluntary daily activity fell to 3388 revolutions and the average daily food consumption increased to almost 20 grams. Table 1 gives the average food consumption and activity for each day of gestation and lactation for the given number of observations. Figure 2 shows these data in graphic form. By consulting table 1 and figure 2 it is readily seen that mating occurred on a peak of the activity, the average run for the twenty-four hours preceding being 16,352 revolutions. After mating the average activity dropped to 2,779 revolutions during the first day of gestation. This great drop in activity was characteristic of all matings and can usually be relied upon as indicating that fertilization has taken place. The second day the activity rose to over 4000 and remained almost constant for three days. As we shall show later, some individuals showed on the fourth day after mating a typical oestrual cycle, which would increase the average. On the fifth day the average again dropped and remained fairly constant until the tenth day when there was another increase which reached a maximum of 4,266 revolutions on the twelfth day. This second rise in the curve was due to oestrus occurring in some of the animals about this time. From this date on there was a fairly uniform reduction in activity to the end of the gestation period when the average run for the last day was 2,072 revolutions. In general there is a very decided drop in activity for the twenty-four hours preceding delivery. Some individual variations to this have been noted in which there was an increase in activity on the day of birth. This is shown in figures 7B and 8A. Table 1 and figure 2 also show that there was a noticeable increase in average activity for the day preceding that of delivery. In some cases

this increase was very marked (fig. 7A) while in other individuals an actual reduction occurred (fig. 8A).

During the first day after delivery there was a decided decrease in average activity to 935 revolutions. This is due to the fact that the mother spends the greater part of this time in nursing and brooding the young. Exceptions to this general behavior are seen in figure 7A, and in several other cases. This again emphasizes the fact that, due to the great individual variation, no definite statement can be made that is characteristic of the rat in regard to its voluntary activity. After the first day of lactation there was a gradual increase in average activity until the fifth day. From this time on to the twentieth day there were only slight and gradual changes. After the twentieth day there was a gradual rise in average activity until the young were weaned.

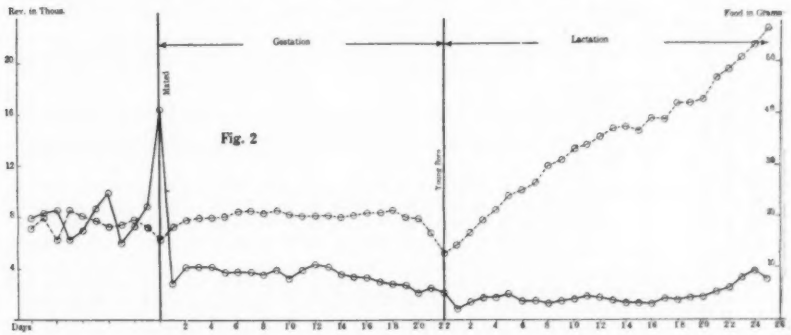


Fig. 2. Graph showing the total average daily activity (solid line) as represented by the number of revolutions voluntarily turned and the average daily food consumption (broken line) during gestation and lactation.

The average food consumption, which prior to mating was approximately 18 grams, decreased for two days and was the lowest (15.5 grams) when oestruation and mating occurred. While there were some exceptions to a decrease in food consumption, just preceding and during oestruation, the majority of individuals behaved in that way. That is, the food consumption showed a four-day rhythm similar to the oestrual rhythm. The low point in the food curve corresponded to oestruation and the high point to dioestrum. This is shown in figure 8B. After mating the amount of food eaten increased until the seventh day of gestation when an average of 21.2 grams was consumed. This amount remained almost constant until the eighteenth day after which it fell during the remaining four days of gestation to an average of 12.9 grams on the day of delivery. Some exceptions to this general decrease in food consumption just prior to delivery have been noted. Some individuals showed an increase in

TABLE 1

*Showing the total and average daily food and activity during successive days of gestation and lactation for the designated number of mothers with litters ranging from 1 to 14 young*

| GESTATION | | | | | | | LACTATION | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | Food | | | Activity | | | Day | Food | | | Activity | | |
| | Number | Total | Average | Number | Total | Average | | Number | Total | Average | Number | Total | Average |
| Mated | 43 | 684 | 15.5 | 55 | 899,356 | 16,352 | | | | | | | |
| 1 | 44 | 794 | 18.0 | 55 | 152,936 | 2,779 | 1 | 44 | 633 | 14.4 | 47 | 43,925 | 935 |
| 2 | 42 | 812 | 19.3 | 55 | 223,913 | 4,071 | 2 | 43 | 714 | 16.6 | 47 | 63,878 | 1,358 |
| 3 | 43 | 844 | 19.6 | 54 | 222,207 | 4,114 | 3 | 44 | 858 | 19.5 | 47 | 76,085 | 1,617 |
| 4 | 46 | 906 | 19.7 | 54 | 225,029 | 4,162 | 4 | 44 | 945 | 21.4 | 47 | 82,331 | 1,750 |
| 5 | 47 | 941 | 20.0 | 55 | 205,169 | 3,728 | 5 | 43 | 1,031 | 24.0 | 46 | 88,856 | 1,930 |
| 6 | 46 | 966 | 21.0 | 55 | 201,513 | 3,663 | 6 | 42 | 1,067 | 25.4 | 45 | 65,719 | 1,455 |
| 7 | 46 | 977 | 21.2 | 55 | 203,159 | 3,694 | 7 | 42 | 1,115 | 26.5 | 45 | 64,929 | 1,441 |
| 8 | 47 | 968 | 20.6 | 55 | 193,294 | 3,514 | 8 | 42 | 1,250 | 29.8 | 45 | 62,420 | 1,296 |
| 9 | 49 | 1,036 | 21.1 | 55 | 212,149 | 3,858 | 9 | 42 | 1,295 | 30.8 | 45 | 70,318 | 1,462 |
| 10 | 44 | 998 | 20.3 | 55 | 177,208 | 3,221 | 10 | 42 | 1,391 | 33.1 | 45 | 73,396 | 1,631 |
| 11 | 49 | 988 | 20.1 | 55 | 210,210 | 3,818 | 11 | 42 | 1,418 | 33.8 | 45 | 83,377 | 1,851 |
| 12 | 49 | 991 | 20.2 | 55 | 234,677 | 4,266 | 12 | 41 | 1,451 | 35.4 | 43 | 73,495 | 1,709 |
| 13 | 49 | 990 | 20.2 | 55 | 223,958 | 4,069 | 13 | 41 | 1,526 | 37.2 | 44 | 67,172 | 1,526 |
| 14 | 49 | 977 | 19.9 | 55 | 194,227 | 3,542 | 14 | 42 | 1,573 | 37.5 | 44 | 61,092 | 1,388 |
| 15 | 49 | 993 | 20.2 | 55 | 184,621 | 3,357 | 15 | 42 | 1,554 | 37.0 | 44 | 55,417 | 1,259 |
| 16 | 49 | 1,026 | 20.9 | 55 | 179,038 | 3,253 | 16 | 41 | 1,602 | 39.0 | 44 | 54,585 | 1,240 |
| 17 | 49 | 1,023 | 20.9 | 55 | 161,005 | 2,928 | 17 | 42 | 1,638 | 39.0 | 44 | 72,590 | 1,649 |
| 18 | 49 | 1,044 | 21.3 | 55 | 151,209 | 2,768 | 18 | 42 | 1,640 | 39.1 | 44 | 65,974 | 1,497 |
| 19 | 49 | 991 | 19.8 | 55 | 144,866 | 2,633 | 19 | 42 | 1,682 | 40.0 | 44 | 74,038 | 1,682 |
| 20 | 49 | 935 | 19.5 | 55 | 112,526 | 2,040 | 20 | 40 | 1,714 | 42.8 | 42 | 75,245 | 1,790 |
| 21 | 49 | 821 | 16.7 | 55 | 132,751 | 2,413 | 21 | 34 | 1,602 | 47.0 | 36 | 79,115 | 2,197 |
| 22 | 26 | 336 | 12.9 | 31 | 64,224 | 2,072 | 22 | 27 | 1,315 | 48.7 | 29 | 72,667 | 2,508 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | 23 | 21 | 1,077 | 51.2 | 23 | 77,734 | 3,378 |
| | | | | | | | 24 | 19 | 1,014 | 53.4 | 21 | 79,814 | 3,800 |
| | | | | | | | 25 | 18 | 1,021 | 56.7 | 19 | 59,200 | 3,115 |
| | | | | | | | 26 | 18 | 986 | 54.8 | 20 | 81,146 | 4,057 |
| | | | | | | | 27 | 18 | 932 | 51.8 | 20 | 109,177 | 5,458 |
| | | | | | | | 28 | 17 | 909 | 53.4 | 19 | 105,679 | 5,559 |
| | | | | | | | 29 | 17 | 1,064 | 62.6 | 19 | 60,211 | 3,165 |
| | | | | | | | 30 | 17 | 1,107 | 65.0 | 18 | 47,589 | 2,642 |
| Total..... | 1,024 | 20,357 | | 1,184 | 4,011,073 | | | 1,007 | 37,124 | | 1,121 | 2,147,174 | |
| Average... | | | 19.9 | | | 3,388 | | | | 36.9 | | | 1,960 |

the amount of food eaten for the two days preceding delivery. However, the amount consumed during the twenty-four hours preceding the birth of the young was considerably less than that which had been eaten during the greater part of gestation. After birth the daily food consumption

TABLE 2

*Showing the total and average daily food and activity during successive days of gestation and lactation for the designated number of mothers with small litters (six and less)*

| GESTATION | | | | | | | LACTATION | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Food | | | Activity | | | | Food | | | Activity | | |
| Day | Number | Total | Average | Number | Total | Average | Day | Number | Total | Average | Number | Total | Average |
| 1 | 16 | 285 | 17.8 | 20 | 53,445 | 2,672 | 1 | 18 | 240 | 13.4 | 20 | 19,698 | 985 |
| 2 | 16 | 315 | 19.7 | 20 | 73,391 | 3,670 | 2 | 18 | 288 | 16.0 | 20 | 30,444 | 1,522 |
| 3 | 16 | 312 | 19.5 | 19 | 75,352 | 3,965 | 3 | 18 | 304 | 16.8 | 20 | 28,067 | 1,403 |
| 4 | 16 | 313 | 19.6 | 19 | 70,016 | 3,681 | 4 | 18 | 346 | 19.2 | 20 | 30,974 | 1,549 |
| 5 | 16 | 332 | 20.7 | 20 | 76,929 | 3,847 | 5 | 17 | 350 | 20.6 | 19 | 34,044 | 1,791 |
| 6 | 16 | 353 | 22.0 | 20 | 72,468 | 3,623 | 6 | 17 | 370 | 21.7 | 19 | 24,455 | 1,286 |
| 7 | 16 | 347 | 21.7 | 20 | 82,137 | 4,107 | 7 | 17 | 374 | 21.8 | 19 | 20,799 | 1,096 |
| 8 | 17 | 348 | 20.5 | 20 | 66,980 | 3,349 | 8 | 17 | 423 | 24.9 | 19 | 22,006 | 1,158 |
| 9 | 18 | 398 | 22.1 | 20 | 79,774 | 3,989 | 9 | 17 | 415 | 24.4 | 19 | 19,489 | 1,026 |
| 10 | 18 | 369 | 20.5 | 20 | 60,911 | 3,045 | 10 | 17 | 463 | 27.2 | 19 | 25,204 | 1,326 |
| 11 | 18 | 354 | 19.7 | 20 | 70,442 | 3,522 | 11 | 17 | 496 | 29.2 | 19 | 27,958 | 1,471 |
| 12 | 18 | 380 | 21.1 | 20 | 74,678 | 3,734 | 12 | 16 | 451 | 28.2 | 18 | 29,048 | 1,614 |
| 13 | 18 | 368 | 20.4 | 20 | 79,016 | 3,951 | 13 | 16 | 460 | 28.8 | 18 | 22,182 | 1,232 |
| 14 | 18 | 364 | 20.2 | 20 | 76,724 | 3,836 | 14 | 17 | 522 | 30.7 | 18 | 18,509 | 1,028 |
| 15 | 18 | 370 | 20.5 | 20 | 68,066 | 3,403 | 15 | 17 | 484 | 28.7 | 18 | 23,671 | 1,316 |
| 16 | 18 | 370 | 20.5 | 20 | 60,609 | 3,030 | 16 | 17 | 544 | 32.0 | 18 | 24,069 | 1,336 |
| 17 | 18 | 370 | 20.5 | 19 | 62,262 | 3,278 | 17 | 17 | 565 | 33.2 | 18 | 30,311 | 1,685 |
| 18 | 18 | 395 | 21.9 | 20 | 60,690 | 3,281 | 18 | 17 | 558 | 32.8 | 18 | 26,273 | 1,458 |
| 19 | 18 | 383 | 21.3 | 20 | 52,833 | 2,642 | 19 | 17 | 527 | 31.0 | 18 | 28,247 | 1,572 |
| 20 | 18 | 367 | 20.4 | 20 | 51,829 | 2,592 | 20 | 16 | 538 | 33.6 | 17 | 29,475 | 1,734 |
| 21 | 18 | 331 | 18.4 | 20 | 48,436 | 2,422 | 21 | 13 | 474 | 36.5 | 14 | 15,489 | 1,107 |
| 22 | 13 | 180 | 13.8 | 15 | 33,254 | 2,217 | 22 | 12 | 438 | 36.5 | 13 | 19,248 | 1,481 |
| | | | | | | | 23 | 8 | 340 | 42.5 | 9 | 26,435 | 2,937 |
| | | | | | | | 24 | 7 | 285 | 40.7 | 8 | 22,572 | 2,821 |
| | | | | | | | 25 | 7 | 330 | 47.1 | 8 | 20,393 | 2,549 |
| Total..... | 376 | 7,604 | | 432 | 1,450,242 | | | 388 | 10,585 | | 426 | 619,060 | |
| Average.... | | | 20.2 | | | 3,208 | | | | 27.3 | | | 1,456 |

increased almost uniformly to an average of 65 grams on the twenty-fifth day of lactation.

*Effect of size of litter.* It was soon seen that activity and food consumption varied with the size of the litter. The data were therefore grouped

into two divisions. Mothers having litters from one to six were classified as small litters and those with seven or more, as large litters. This arbitrary division was chosen because it was nearest the mean between the two extremes, which were one and fourteen. The average activity

TABLE 3

*Showing the total and average daily food and activity during successive days of gestation and lactation for the designated number of mothers with large litters (seven and over)*

| GESTATION | | | | | | | LACTATION | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | Food | | | Activity | | | Day | Food | | | Activity | | |
| | Number | Total | Average | Number | Total | Average | | Number | Total | Average | Number | Total | Average |
| 1 | 22 | 398 | 18.1 | 26 | 98,634 | 3,792 | 1 | 25 | 382 | 15.3 | 26 | 24,225 | 932 |
| 2 | 22 | 402 | 18.2 | 26 | 125,944 | 4,884 | 2 | 25 | 432 | 17.3 | 26 | 33,519 | 1,289 |
| 3 | 22 | 407 | 18.5 | 26 | 118,474 | 4,556 | 3 | 25 | 543 | 21.7 | 26 | 50,478 | 1,938 |
| 4 | 24 | 488 | 20.3 | 26 | 123,626 | 4,752 | 4 | 25 | 586 | 23.4 | 26 | 50,164 | 1,927 |
| 5 | 24 | 466 | 19.4 | 26 | 98,432 | 3,787 | 5 | 25 | 672 | 26.9 | 26 | 49,341 | 1,897 |
| 6 | 24 | 498 | 20.7 | 26 | 89,063 | 3,427 | 6 | 25 | 666 | 26.6 | 26 | 41,264 | 1,587 |
| 7 | 24 | 505 | 21.0 | 26 | 95,345 | 3,663 | 7 | 25 | 741 | 29.6 | 26 | 44,179 | 1,699 |
| 8 | 25 | 519 | 20.8 | 26 | 94,872 | 3,649 | 8 | 25 | 826 | 33.0 | 26 | 40,414 | 1,553 |
| 9 | 25 | 502 | 20.1 | 26 | 103,271 | 3,970 | 9 | 15 | 880 | 35.2 | 26 | 50,824 | 1,955 |
| 10 | 25 | 501 | 20.0 | 26 | 89,519 | 3,442 | 10 | 25 | 928 | 37.1 | 26 | 48,192 | 1,853 |
| 11 | 25 | 526 | 21.0 | 26 | 111,271 | 4,280 | 11 | 25 | 922 | 36.9 | 26 | 55,419 | 2,129 |
| 12 | 25 | 505 | 20.0 | 26 | 124,728 | 4,800 | 12 | 25 | 1,000 | 40.0 | 26 | 45,863 | 1,760 |
| 13 | 25 | 496 | 19.8 | 26 | 114,668 | 4,409 | 13 | 25 | 1,066 | 42.7 | 26 | 44,990 | 1,731 |
| 14 | 25 | 499 | 20.0 | 26 | 88,406 | 3,394 | 14 | 25 | 1,051 | 42.0 | 26 | 42,603 | 1,637 |
| 15 | 25 | 495 | 19.8 | 26 | 83,265 | 3,201 | 15 | 25 | 1,070 | 42.8 | 26 | 31,746 | 1,221 |
| 16 | 25 | 531 | 21.2 | 26 | 86,795 | 3,338 | 16 | 25 | 1,070 | 42.8 | 26 | 30,512 | 1,173 |
| 17 | 25 | 531 | 21.2 | 26 | 70,636 | 2,714 | 17 | 25 | 1,073 | 42.9 | 26 | 42,479 | 1,625 |
| 18 | 25 | 503 | 20.1 | 26 | 61,524 | 2,364 | 18 | 25 | 1,082 | 43.3 | 26 | 44,701 | 1,715 |
| 19 | 25 | 482 | 19.3 | 26 | 64,707 | 2,481 | 19 | 25 | 1,155 | 46.2 | 26 | 45,791 | 1,761 |
| 20 | 25 | 440 | 17.6 | 26 | 38,813 | 1,491 | 20 | 24 | 1,176 | 49.0 | 25 | 45,770 | 1,829 |
| 21 | 25 | 372 | 14.9 | 26 | 62,039 | 2,383 | 21 | 21 | 1,128 | 53.6 | 22 | 63,626 | 2,892 |
| 22 | 8 | 94 | 11.8 | 8 | 8,776 | 1,097 | 22 | 15 | 877 | 58.5 | 16 | 53,419 | 3,339 |
| | | | | | | | 23 | 13 | 737 | 56.7 | 14 | 51,299 | 3,661 |
| | | | | | | | 24 | 12 | 729 | 60.7 | 13 | 57,242 | 4,405 |
| | | | | | | | 25 | 11 | 691 | 62.8 | 11 | 38,807 | 3,529 |
| Total..... | 542 | 10,160 | | 580 | 1,952,808 | | | 556 | 21,483 | | 579 | 1,075,878 | |
| Average.... | | | 18.7 | | | 3,367 | | | | 38.6 | | | 1,858 |

and food consumption for each day of gestation and lactation for small litters is given in table 2 and for large litters in table 3. These two tables are shown in graphic form in figures 3 and 4 respectively.

Table 2 shows that during gestation the mothers with small litters had an average daily run of 3208 revolutions and an average daily food consumption of 20.2 grams. Table 3 shows that the mothers with large litters during the gestation period averaged 3367 revolutions daily and

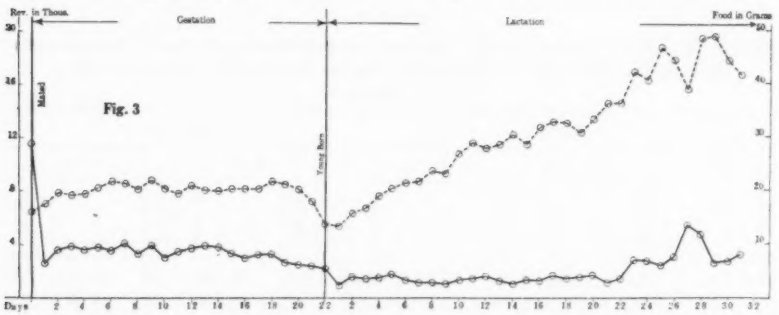


Fig. 3. Graph showing the average daily activity (solid line) and the average daily food consumption (broken line) of mothers with small litters (six or less).

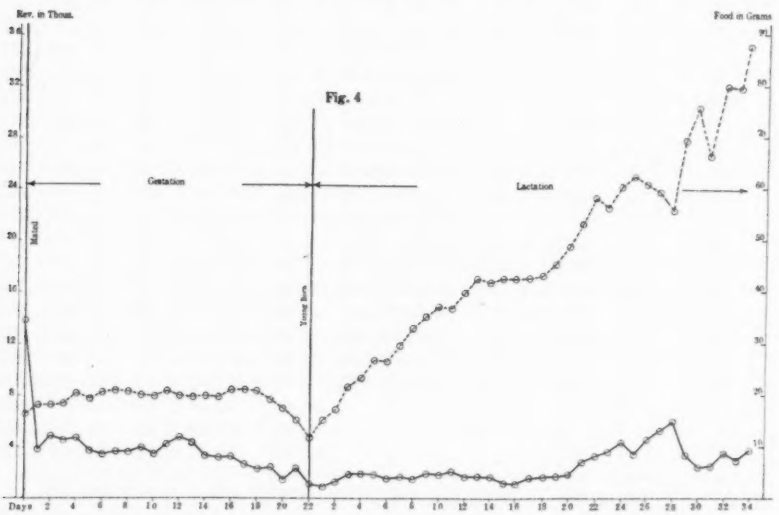


Fig. 4. Graph showing the average daily activity (solid line) and the average daily food consumption (broken line) of mothers with large litters (seven or greater).

consumed an average of 18.7 grams of food daily. If these results are compared we see that the mothers with small litters did less voluntary running, ate more food and produced fewer young than the mothers with large litters. This is a physiological paradox which we are at present

unable to explain. The age of the mothers could have had little influence since the average age of the mothers at birth of the small litters and large litters was 225 and 211 days respectively. The gestation periods of these two groups as shown in figures 3 and 4, are quite similar and conform closely to that of the total average as shown in figure 2.

We would naturally expect a marked difference in the results of the two groups during lactation. The mothers with small litters averaged to run daily 1456 revolutions during the twenty-five days of lactation and to consume an average of 27.3 grams of food daily. Those with large litters averaged 1858 revolutions and 38.6 grams of food daily. An increase of activity in the large litter group over the small litter group is again noticed during lactation. The range of average food consumption for small litters during the twenty-five days of lactation was from 13.4 grams on the first day to 47.1 grams on the twenty-fifth day. In large litters the range was from 15.3 grams on the first day to 62.8 grams on the twenty-fifth day. The increase in food consumption during the first fifteen or twenty days was due to the increased lactation on the part of the mother. After this time the young began to leave the nest and to eat. The amount the young ate increased daily from this time on until they were weaned. The marked increase in food consumption, beginning about the twentieth day of lactation, was therefore due to the combined consumption by both mother and young. We have as yet not been able to devise a means of getting the food consumption of the mother during the days of lactation extending beyond about the twentieth day. The tendency of the young to leave the nest and get onto the revolving cage interfered with the normal voluntary activity of the mother. The larger the litter the greater was the handicap for the mother. Yet in spite of this greater interference the mothers of large litters had a greater average daily run, both during gestation and lactation. This cannot be explained by assuming an individual tendency on the part of mothers with large litters for greater activity for most of the mothers had both large and small litters in the several litters they delivered. Since the food was always the same and in abundant amounts and the environment as nearly constant as it was possible to make it, the only assumption which seems reasonable to us that would account for the differences is that there may have been changes in the vitality of the different individuals at various times. The lower the vitality the fewer would be the young produced and less the tendency for running, while with vigorous animals the reverse would occur. Later investigation may throw more light on this subject.

*Effect of size of litter on length of gestation.* In order to show the effect of the size of the litter on the gestation time and the variations exhibited by different individuals we have arranged the data as shown in tables 4, 5, 6 and 7. Tables 4 and 5 are for the gestation period and tables 6

and 7 the lactation period of mothers with small and large litters respectively. Tables 4 and 5 give the age of the mother at birth of the designated litter, the number of the litter, the size of the litter, the number of each sex, the gestation time, and the total and average daily food consumption and activity during the period of gestation.

TABLE 4

*Showing the age of the mother at birth of designated litter, the size and sexes of the litter, the gestation period, the total and average daily food consumption and the total and average daily activity during gestation of mothers with small litters*

| FEMALE | | LITTER | | | | GESTATION | | FOOD CONSUMED | | ACTIVITY | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Number | Age | Number | Size | Males | Females | Days | Hours | Total food | Daily average | Total activity | Daily average |
| B-1 | 236 | 1 | 4 | 2 | 2 | 22 | 1 | 513 | 24.4 | 42,404 | 2,019 |
| B-1 | 296 | 2 | 1 | 1 | | 23 | 5 | 486 | 21.1 | 26,010 | 1,131 |
| B-2 | 225 | 1 | 5 | 3 | 2 | 21 | 5 | 526 | 25.0 | 49,497 | 2,357 |
| B-3 | 283 | 3 | 4 | 1 | 3 | 22 | 4 | 478 | 21.7 | 61,961 | 2,816 |
| B-5 | 249 | 1 | 3 | 2 | 1 | 21 | 23 | 481 | 21.9 | 18,031 | 820 |
| C-1 | 226 | 2 | 4 | 4 | | 22 | 2 | 416 | 19.8 | 82,803 | 4,140 |
| C-1 | 276 | 3 | 5 | 2 | 3 | 21 | 10 | 384 | 18.6 | 52,377 | 2,494 |
| C-2 | 279 | 2 | 6 | 2 | 4 | 22 | | 398 | 18.1 | 54,263 | 2,466 |
| C-3 | 226 | 1 | 4 | 1 | 3 | 22 | | 454 | 20.6 | 89,472 | 4,067 |
| C-3 | 277 | 2 | 5 | 2 | 3 | 22 | | 433 | 19.7 | 53,974 | 2,453 |
| C-4 | 254 | 2 | 4 | 3 | 1 | 22 | 5 | 389 | 17.4 | 49,403 | 2,247 |
| D-3 | 172 | 2 | 3 | 2 | 1 | 21 | 23 | 504 | 22.9 | 98,607 | 4,482 |
| D-4 | 224 | 2 | 6 | 3 | 3 | 21 | 5 | 477 | 22.7 | 40,760 | 1,955 |
| D-5 | 237 | 2 | 5 | 2 | 3 | 21 | 18 | 475 | 21.6 | 65,665 | 2,976 |
| E-1 | 173 | 1 | 3 | 2 | 1 | 22 | 2 | 481 | 21.9 | 36,256 | 1,648 |
| E-5 | 195 | 1 | 6 | 2 | 4 | 22 | 2 | 354 | 16.1 | 85,720 | 3,896 |
| F-1 | 236 | 2 | 3 | 3 | | 21 | 22 | 416 | 18.9 | 33,412 | 1,518 |
| F-2 | 222 | 2 | 3 | 1 | 2 | 22 | 6 | 418 | 19.0 | 108,806 | 4,946 |
| F-4 | 132 | 1 | 5 | 4 | 1 | 22 | 20 | | | 185,898 | 8,450 |
| F-4 | 243 | 3 | 4 | 1 | 3 | 22 | 4 | 364 | 16.5 | 93,668 | 4,257 |
| F-5 | 115 | 1 | 4 | 3 | 1 | 22 | | | | 119,592 | 5,436 |
| F-5 | 171 | 2 | 5 | 1 | 4 | 22 | | 350 | 15.9 | 88,267 | 4,012 |
| F-5 | 221 | 3 | 6 | 3 | 3 | 22 | 2 | 363 | 16.5 | 33,774 | 1,535 |
| Total..... | 5,168 | | 98 | 50 | 48 | | | 10,435 | 4,203 | 1,540,620 | 72,121 |
| Average.... | 225 | | 4.25 | 2.17 | 2.09 | 21 | 23.8 | 497 | 20 | 66,983 | 3,131 |

Table 4 shows that the average age of the mothers with small litters was 225 days at birth of the litter, that the average size of the litter was 4.25, that the males slightly surpassed the females in number, and that the average gestation time was twenty-one days and 23.8 hours. The shortest gestation period was twenty-one days and five hours and the longest was twenty-two days and twenty hours.

Table 5 shows that the average age of the females which produced large litters was 211.3 days at the birth of the litter, that the average size of the litter was 9.12, that the number of males born was slightly less than that of the females, and that the average duration of gestation was twenty-one

TABLE 5

*Showing the age of the mother at birth of designated litter, the size and sexes of the litter, the gestation period, the total and average daily food consumed and the total and average daily activity during gestation of mothers with large litters*

| FEMALE | | LITTER | | | | GESTATION | | FOOD CONSUMED | | ACTIVITY | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Number | Age | Number | Size | Males | Females | Days | Hours | Total food | Daily average | Total activity | Daily average |
| B 2 | 275 | 2 | 9 | 5 | 4 | 22 | | 492 | 22.2 | 26,564 | 1,207 |
| B 3 | 235 | 2 | 12 | 6 | 6 | 21 | 8 | 449 | 20.4 | 74,732 | 3,400 |
| B 4 | 270 | 2 | 9 | 4 | 5 | 22 | 2 | 485 | 22.1 | 47,430 | 2,156 |
| C 2 | 230 | 1 | 11 | 3 | 8 | 21 | 5 | 429 | 20.4 | 78,301 | 3,728 |
| C 4 | 181 | 1 | 14 | 8 | 6 | 21 | | | | 109,315 | 5,205 |
| C 4 | 295 | 3 | 7 | 2 | 5 | 22 | 2 | 383 | 17.3 | 78,362 | 3,562 |
| C 5 | 260 | 1 | 10 | 4 | 6 | 21 | 5 | 368 | 17.5 | 49,662 | 2,365 |
| D 1 | 162 | 1 | 10 | 6 | 4 | 21 | 2 | 453 | 21.6 | 90,561 | 4,312 |
| D 1 | 213 | 2 | 7 | 4 | 3 | 21 | 9 | 389 | 18.5 | 73,232 | 3,487 |
| D 2 | 174 | 1 | 7 | 3 | 4 | 21 | 5 | 372 | 16.9 | 121,562 | 5,525 |
| D 2 | 218 | 2 | 9 | 5 | 4 | 21 | 5 | 356 | 16.3 | 82,190 | 3,740 |
| D 3 | 219 | 3 | 11 | 5 | 6 | 21 | 4 | 500 | 23.8 | 53,719 | 2,558 |
| D 4 | 175 | 1 | 10 | 5 | 5 | 21 | 3 | 438 | 20.8 | 66,992 | 3,190 |
| D 5 | 172 | 1 | 8 | 3 | 5 | 21 | 1 | 461 | 22.0 | 92,962 | 4,427 |
| E 1 | 241 | 2 | 8 | 4 | 4 | 21 | 4 | 461 | 22.0 | 38,075 | 1,813 |
| E 2 | 181 | 1 | 8 | 4 | 4 | 21 | 8 | 373 | 17.7 | 82,666 | 3,936 |
| E 2 | 229 | 2 | 8 | 3 | 5 | 22 | 2 | 403 | 18.3 | 74,168 | 3,367 |
| E 3 | 181 | 1 | 11 | 5 | 6 | 21 | 8 | 344 | 16.8 | 77,495 | 3,522 |
| E 3 | 230 | 2 | 10 | 5 | 5 | 21 | 9 | 364 | 17.6 | 76,645 | 3,650 |
| E 4 | 182 | 1 | 9 | 4 | 5 | 22 | | 351 | 16.7 | 76,325 | 3,634 |
| E 5 | 240 | 2 | 7 | 3 | 4 | 22 | | 428 | 19.4 | 63,856 | 2,902 |
| F 1 | 188 | 1 | 8 | 7 | 1 | 22 | | 415 | 18.8 | 32,325 | 1,469 |
| F 2 | 174 | 1 | 9 | 5 | 4 | 21 | 4 | 394 | 18.8 | 95,671 | 4,555 |
| F 3 | 163 | 2 | 11 | 7 | 4 | 21 | 4 | 512 | 24.2 | 84,342 | 4,016 |
| F 3 | 226 | 3 | 7 | 2 | 5 | 21 | 10 | 512 | 24.4 | 96,273 | 4,584 |
| F 4 | 180 | 2 | 7 | 4 | 3 | 21 | 4 | 344 | 16.7 | 93,862 | 4,469 |
| Total..... | 5,494 | | 237 | 116 | 121 | | | 10,476 | 491.2 | 1,937,287 | 90,779 |
| Average.... | 211.3 | | 9.12 | 4.46 | 4.66 | 21 | 11.38 | 419 | 19.65 | 74,511 | 3,491 |

days and 11.38 hours. The shortest gestation period was twenty-one days, which resulted in fourteen young, and the longest was twenty-two days (three litters) followed by the birth of seven, eight and nine young. We have recently demonstrated a much shorter period of gestation in

another experiment now in progress in which another group of females is being maintained in the same environment and on the same food formula. One female, whose age at delivery was 126 days, had a litter of ten young following a gestation of twenty days and six hours' duration. Another had twelve young at the age of 107 days following a gestation period of twenty days five and one-half hours. From these two cases one might conclude that the younger the animal the shorter would be its period of gestation. But this does not necessarily follow for another female delivered eleven young at the age of 131 days after a gestation period of twenty-one days and five hours. With two other young females, which had small litters, one delivered five young at the age of 132 days and had a gestation period of twenty-one days, while the other delivered six at the age of 137 days and had a gestation period of twenty-one days one and three-fourths hours. All these results seem to indicate that in general the larger the litter, the shorter will be the gestation period and that this shortening of gestation is to some extent correlated with the age of the mother, young rats having in general a shorter gestation period than older animals. These results also show that there is a great individual variation which plays an important part. It is also interesting to note that the average weight of the young of small litters was but a small fraction of a gram heavier than that of large litters. This small difference in weight might easily be accounted for by the difference in the true age, or the actual time since fertilization. That is, if the gestation period of one litter should be twenty-one days and that of a second litter twenty-two days, the second litter at birth would be one day farther along in its development than the first and should as a result average to weigh heavier.

One explanation seems reasonable to us as a possible cause for a shorter gestation period for the large litters. We have noticed with mothers carrying large litters that near the end of gestation period their abdominal wall was very tense and the skin taut. This ever-increasing tension, due to the rapid growth of the young during the last six or seven days of intra-uterine life, may be a contributing factor to the onset of early labor, or possible abortion. The weight of the eleven and twelve young and accessory structures of litters delivered by two of our young females was 32 per cent of the weight of the mothers the day after delivery: another litter of ten was 29 per cent of the weight of the mother; while litters of five and six were but 15 per cent of the weight of the mothers. In older mothers the percentage loss is not so great. This accords with the slight lengthening of the gestation period as rats grow older. This appears to be generally true of both small and large litters.

These results show that, though there was marked individual variation exhibited by different mothers, large litters generally shortened the period

of gestation, that the effect was more pronounced in young animals than in older ones, and again emphasizes the fact that the mothers with large litters performed more work and developed a greater number of young on less food consumption than mothers with small litters.

*Effect of size of litter during lactation.* Tables 6 and 7 show that there was great individual variation in food consumption and voluntary activity exhibited by different mothers. In general, however, a fairly close cor-

TABLE 6

*Showing the age of the mother at birth of designated litter, the size of the litter, and the sexes born and nursed, the total and average daily food consumed and the total and average daily activity during the first twenty days of lactation of mothers with small litters*

| FEMALE | | YOUNG BORN | | | | YOUNG NURSED | | | FOOD CONSUMED | | ACTIVITY | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number | Age | Litter | Total | Male | Female | Total | Male | Female | Total | Daily average | Total | Daily average |
| B 1 | 236 | 1 | 4 | 2 | 2 | 4 | 2 | 2 | 466 | 23.3 | 25,889 | 1,494 |
| B 2 | 225 | 1 | 5 | 3 | 2 | 4 | 2 | 2 | 633 | 31.6 | 29,457 | 1,473 |
| B 3 | 283 | 3 | 4 | 1 | 3 | 3 | 1 | 2 | 497 | 24.9 | 25,652 | 1,283 |
| B 5 | 249 | 1 | 3 | 2 | 1 | 3 | 2 | 1 | 490 | 24.5 | 16,293 | 815 |
| C 1 | 226 | 2 | 4 | 4 | | 4 | 4 | | 485 | 24.3 | 13,550 | 677 |
| C 1 | 276 | 3 | 5 | 2 | 3 | 5 | 2 | 3 | 520 | 26.0 | 5,244 | 262 |
| C 2 | 279 | 2 | 6 | 2 | 4 | 6 | 2 | 4 | 569 | 28.5 | 22,211 | 1,111 |
| C 3 | 226 | 1 | 4 | 1 | 3 | 4 | 1 | 3 | 502 | 25.1 | 12,129 | 606 |
| C 3 | 277 | 2 | 5 | 2 | 3 | 5 | 2 | 3 | 506 | 25.3 | 14,941 | 747 |
| D 3 | 172 | 2 | 3 | 2 | 1 | 3 | 2 | 1 | 512 | 25.6 | 45,791 | 2,289 |
| E 1 | 173 | 1 | 3 | 2 | 1 | 2 | 1 | 1 | 419 | 21.0 | 17,481 | 874 |
| E 5 | 195 | 1 | 6 | 2 | 4 | 6 | 2 | 4 | 533 | 26.7 | 38,957 | 1,948 |
| F 2 | 222 | 2 | 3 | 1 | 2 | 2 | | 2 | 386 | 19.3 | 23,102 | 1,155 |
| F 4 | 132 | 1 | 5 | 4 | 1 | 5 | 4 | 1 | | | 43,632 | 2,182 |
| F 5 | 115 | 1 | 4 | 3 | 1 | 4 | 3 | 1 | | | 63,246 | 3,162 |
| F 5 | 171 | 2 | 5 | 1 | 4 | 5 | 1 | 4 | 497 | 24.9 | 14,153 | 708 |
| F 5 | 221 | 3 | 6 | 3 | 3 | 6 | 3 | 3 | 573 | 28.7 | 21,251 | 1,063 |
| Total..... | 3,978 | | 75 | 37 | 38 | 71 | 34 | 37 | 7,588 | 379.7 | 432,979 | 21,848 |
| Average... | 234 | | 4.3 | 2.18 | 2.24 | 4.17 | 2 | 2.17 | 506 | 25.3 | 25,469 | 1,285 |

relation can be made between the amount of food eaten and the number of young nursed. As would be expected the average daily food consumption for mothers with small litters was less than that for large litters, being 25.3 and 34.57 grams respectively. The lowest food intake for the twenty days of lactation was shown by rat F2 which ate 386 grams and nursed two young. This same rat, while nursing an earlier litter of nine young, consumed 688 grams during twenty days. A portion of this food was

utilized by the mother in growth. While nursing the nine young she gained 14 grams in body weight. While nursing the later litter of two young she had reached adult size and actually lost 3 grams during the lactation period. She also ate one of the three young born in this litter which was another factor in lowering her weighed food consumption.

TABLE 7

*Showing the age of the mother at birth of designated litter, the size of the litter and the sexes born and nursed, the total and average daily food consumed and the total and average daily activity during the first twenty days of lactation of mothers with large litters*

| FEMALE | | YOUNG BORN | | | | YOUNG NURSED | | | FOOD CONSUMED | | ACTIVITY | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number | Age | Litter | Total | Male | Female | Total | Male | Female | Total | Daily average | Total | Daily average |
| B 2 | 275 | 2 | 9 | 5 | 4 | 9 | 5 | 4 | 794 | 39.7 | 26,073 | 1,304 |
| B 3 | 235 | 2 | 12 | 6 | 6 | 12 | 6 | 6 | 805 | 40.2 | 42,352 | 2,118 |
| B 4 | 270 | 2 | 9 | 4 | 5 | 9 | 4 | 5 | 805 | 40.2 | 48,345 | 2,417 |
| C 2 | 230 | 1 | 11 | 3 | 8 | 11 | 3 | 8 | 745 | 37.2 | 23,708 | 1,185 |
| C 4 | 181 | 1 | 14 | 8 | 6 | 9 | 6 | 3 | | | 67,746 | 3,387 |
| C 5 | 260 | 1 | 10 | 4 | 6 | 10 | 4 | 6 | 641 | 32.0 | 16,135 | 807 |
| D 1 | 162 | 1 | 10 | 6 | 4 | 10 | 6 | 4 | 735 | 36.7 | 41,657 | 2,083 |
| D 1 | 213 | 2 | 7 | 4 | 3 | 7 | 4 | 3 | 633 | 31.7 | 21,402 | 1,070 |
| D 2 | 174 | 1 | 7 | 3 | 4 | 7 | 3 | 4 | 560 | 18.0 | 42,498 | 2,125 |
| D 2 | 218 | 2 | 9 | 5 | 4 | 9 | 5 | 4 | 630 | 31.5 | 27,484 | 1,374 |
| D 3 | 219 | 3 | 11 | 5 | 6 | 11 | 5 | 6 | 759 | 38.0 | 45,478 | 2,274 |
| D 4 | 175 | 1 | 10 | 5 | 5 | 10 | 5 | 5 | 777 | 38.8 | 41,310 | 2,065 |
| D 5 | 172 | 1 | 8 | 3 | 5 | 8 | 3 | 5 | 657 | 32.9 | 43,521 | 2,176 |
| E 2 | 181 | 1 | 8 | 4 | 4 | 7 | 3 | 4 | 632 | 31.6 | 25,797 | 1,290 |
| E 3 | 181 | 1 | 11 | 5 | 6 | 11 | 5 | 6 | 666 | 33.3 | 29,381 | 1,469 |
| E 4 | 182 | 1 | 9 | 4 | 5 | 9 | 4 | 5 | 692 | 34.6 | 18,893 | 945 |
| F 1 | 188 | 1 | 8 | 7 | 1 | 8 | 7 | 1 | 626 | 31.3 | 27,246 | 1,362 |
| F 2 | 174 | 1 | 9 | 5 | 4 | 9 | 5 | 4 | 688 | 34.4 | 18,714 | 926 |
| F 3 | 163 | 2 | 11 | 7 | 4 | 11 | 7 | 4 | 956 | 47.8 | 25,340 | 1,267 |
| F 3 | 226 | 3 | 7 | 2 | 5 | 7 | 2 | 5 | 701 | 35.0 | 49,983 | 2,499 |
| F 4 | 180 | 2 | 7 | 4 | 3 | 7 | 4 | 3 | 530 | 26.5 | 6,856 | 343 |
| Total..... | 4,259 | | 197 | 99 | 98 | 191 | 96 | 95 | 14,032 | 691.4 | 689,919 | 34,486 |
| Average.. | 203 | | 9.4 | 4.7 | 4.67 | 9.1 | 4.57 | 4.52 | 702 | 34.57 | 32,853 | 1,642 |

The greatest food consumption was by rat F3 which consumed 956 grams during the twenty days of lactation while nursing eleven young. This was also a growing animal and gained 10 grams in weight during lactation. B3, an almost mature rat, nursed twelve young and ate during the twenty days of lactation 805 grams. During this time she showed a slight gain in weight.

Tables 6 and 7 also show that in the total young of small litters three males and one female died and were eaten, while in the large litters three males and three females were eaten. Expressed in per cent of the number born this is equivalent to 8.1 per cent males and 2.6 per cent females in the small litters and practically 3 per cent for each sex in the large litters. This lower per cent of deaths indicates that the young of large litters had a stronger vitality than those of small litters.

It will also be noted by a study of these tables that the amount of activity shown has a direct bearing on the amount of food consumed. One example will suffice. By comparing the activity and food consumption of the mothers in table 7 which had litters of seven young it will be noticed that F4, which had the lowest total food consumption (530 grams), had also the lowest total voluntary activity (6856 revolutions). It will also be seen that F3, which had the greatest total food consumption (701 grams) had also the greatest total activity (49983). The other mothers nursing seven young show intermediate positions between these two extremes. If these five rats are arranged in the order of their food consumption they would be F4, D2, E2, D1 and F3; but if placed in the order of voluntary activity they would have the order of F4, D1, E2, D2 and F3. F4, E2 and F3 occupy the same relative positions under both arrangements while D1 and D2 show how individual variations may modify general averages.

From these results we may conclude that during lactation the food consumption of the mother depends in general on the number of young nursed, the amount of voluntary activity performed, and on the age of the mother; that is, whether she has reached her adult size or is still growing.

*Oestruation during gestation.* In many cases we have observed that oestruation was completely suppressed during gestation. This accords with the general results of other investigators. Though this was quite common and was true of over 10 per cent of the animals studied, we have found a large number of instances where one or more oestrual cycles seem to have occurred during the gestation period. Since oestruation is coincident with high peaks in the curves of voluntary activity it is easy to recognize when they happened by studying the plotted curves of the individual rats. Figure 7B shows a high point in the voluntary activity of rat D2 on the twelfth day of gestation. Figure 8A shows a similar abrupt high run of rat D1 also on the twelfth day of gestation. Other cases were noted in which a spurt of activity occurred on the twelfth day. One rat (E5) showed a series of almost rhythmical fluctuations in its voluntary activity with almost uniform intervals of time. The spurts of activity cited in these different cases we think represent true oestrus. No mating tests were made at these times to verify our assumption as they would

have interfered with the experiment. In experiments now in progress we are finding similar spurts of voluntary activity which occur during gestation and are testing these times to determine if they represent true oestruation by mating tests and vaginal smears. We have, however, a record of one animal in which oestruation did occur during gestation. Its history is as follows: A male and a female rat were placed together in a stationary cage February 2, 1924. The male was removed and the female isolated February 23, or twenty-one days after mating. One day after isolation (February 24) she delivered five young all of which were eaten within three days. Isolation of the female continued and on March 10, 1924, fifteeen days after birth of the litter of five, she gave birth to a second litter of six which she reared. Since the only chance for copulation was during the twenty-one days that the male and female were together, oestruation, copulation and fertilization must have occurred during the first gestation period. Just what effect, if any, the carrying of young had on the length of the second gestation period or the fertilization of the second litter on the first gestation period is not known. But if each of these two periods was twenty-two days then oestruation occurred on the fifteenth day of the first gestation period. This was a few days later than the cases described above.

The frequency in which oestruation occurred, as indicated by spurts of activity, during gestation and lactation following our fifty-six matings, is shown in table 8. This table gives the age of the mother at mating, the number of young born, nursed, and age at weaning, and the days after mating and delivery on which the spurts of activity occurred. It shows the marked individual variation which existed.

Eleven of these fifty-six gestation periods showed no spurts of activity, thus indicating complete suppression of oestrus. A total of 114 high points in activity was found in the remaining forty-five gestation periods. These showed from one to as many as six marked increases in voluntary activity in different cases. Of the total number observed eighty-seven occurred during the first thirteen days and one hundred and nine during the first seventeen days. If these are grouped according to successive days we find that 20 per cent occurred during the second, third and fourth days, 19 per cent during the seventh, eighth and ninth days, 25 per cent during the eleventh, twelfth and thirteenth days, and 15 per cent during the fifteenth, sixteenth and seventeenth days. In other words the frequency of occurrence showed that the spurts of activity tended to a rhythmic grouping around the fourth, eighth, twelfth and sixteenth days of gestation, thus showing a four-day rhythm similar to that of the non-pregnant female. More spurts of activity occurred on the twelfth and thirteenth days than on any other days during the gestation period. Since this corresponds closely to the time oestrual cycles are reëstab-

TABLE 8

*Giving the age of the mother at mating, the number and size of the litter born, the number of young nursed and their age at weaning. The high points in activity (indicating oestrus) are recorded on the days they occurred after mating (during gestation) and after delivery (during lactation and after weaning). B indicates a high point at birth of litter, C a subsequent mating, and E, young eaten*

| RAT | | LITTER | | GESTATION | LACTATION | | |
|---|---|---|---|---|---|---|---|
| | | | | | Young weaned | | |
| Number | Age at mating | Number | Young | Oestrus on ? day | Number | Age | Oestrus ? days after delivery |
| B1 | 214 | 1 | 4 | 3 | 4 | 22 | 25, 37 (C) |
| B1 | 273 | 2 | 1 | | E | | 13, 18, 23, 28, 32, 36 |
| B2 | 204 | 1 | 5 | 2 | 4 | 22 | 1, 3, 5, 23, 28, (C) |
| B2 | 253 | 2 | 9 | 12, 16 | 9 | 34 | 1, 21, 27, 36, 46 |
| B3 | 155 | 1 | E | 6, 12 | E | | 3, 10, 13, 16, 19, 23, 28, 32, 36 (C) |
| B3 | 214 | 2 | 12 | 2, 9, 12, 14 | 12 | 22 | 1, 5, 11, 15, 18, 22, 26 (C) |
| B3 | 271 | 3 | 4 | | 4 | 31 | B, 2, 7, 13, 17, 21, 27, 33, 37, 41, 45 |
| B4 | 212 | 1 | E | | E | | 7, 11, 15 (C), 19 |
| B4 | 248 | 2 | 9 | 4 | 9 | 34 | 4, 9, 14, 24, 28, 32, 37, 41, 44, 48, 52 |
| B5 | 227 | 1 | 4 | | 3 | 20 | 27, 30, 38, 42 |
| B1 | 155 | 1 | E | 2, 8, 15 | E | | 14, 18, 22, 26 (C) |
| C1 | 204 | 2 | 4 | 5, 13, 17 | 4 | 22 | 19, 25, 29 (C) |
| C1 | 255 | 3 | 5 | 3 | 5 | 20 | 22, 26, 30, 34, 38 |
| C2 | 209 | 1 | 11 | 2, 8, 13 | 11 | 24 | 5, 22, 27 (C) |
| C2 | 259 | 2 | 6 | | 6 | 30 | 18, 21, 25, 29, 33, 37 |
| C3 | 204 | 1 | 4 | 7, 13, 18 | 4 | 22 | 18, 25, 29 (C) |
| C3 | 255 | 2 | 5 | 13 | 5 | 20 | 18, 23, 26, 31, 35 |
| C4 | 160 | 1 | 14 | 4, 9, 12, 16 | 9 | 30 | B, 4?, 8, 12, 19, 29, 35, 38, 42, 44 (C) |
| C4 | 232 | 2 | 4 | 13 | E | | 4, 15, 19 (C) |
| C4 | 273 | 3 | 7 | 3, 7, 11, 14 | 7 | 30 | 15, 23, 28, 33, 37, 41 |
| C5 | 239 | 1 | 10 | | 10 | 21 | 13, 24, 28 (C) |
| C5 | 288 | 2 | E | | E | | 1?, 9, 14, 18, 22, 24 |
| D1 | 141 | 1 | 10 | 6, 10, 16 | 10 | 22 | B, 11, 13, 26, 30 (C) |
| D1 | 192 | 2 | 7 | 12 | 7 | 33 | 7, 26, 30 |
| D2 | 153 | 1 | 7 | | 7 | 21 | 2?, 21 (C) |
| D2 | 196 | 2 | 9 | 12 | 9 | 32 | B, 6, 10, 14, 18, 24, 29, 32, 37, 41, 45 |
| D3 | 93 | 1 | E | 9, 12 | E | | 7, 12, 14, 18, 21, 23, 25, 29, 35 (C) |
| D3 | 150 | 2 | 3 | 5, 14 | 3 | 20 | 6, 12, 22, 26 (C) |
| D3 | 198 | 3 | 11 | 4, 9, 13 | 11 | 32 | B, 4, 8, 15, 21, 26, 30, 34, 38 |
| D4 | 154 | 1 | 10 | | 10 | 21 | B, 4, 11, 17, 24, 28 (C) |
| D4 | 203 | 2 | 6 | | 5 | 30 | B, 3, 12, 17, 31, 35, 39 |
| D5 | 151 | 1 | 8 | 4, 11 | 8 | 20 | B, 3, 7, 17, 19, 23, 29, 35, 39, 43 (C 44) |
| D5 | 216 | 2 | 5 | 5, 9, 12, 15 | 5 | 30 | 12, 16, 20, 25, 30 |
| E1 | 151 | 1 | 3 | 4 | 2 | 22 | 25, 36, 47 (C) |
| E1 | 220 | 2 | 8 | | 8 | 30 | 3, 7, 11, 14, 20, 34, 38, 42, 46 |
| E2 | 160 | 1 | 8 | 2, 5, 11, 16, 19 | 7 | 21 | 4, 11, 18, 22, 26 (C) |
| E2 | 207 | 2 | 8 | 4, 8, 14 | 8 | 30 | 6, 12, 17, 21, 23, 26, 28, 32 |

TABLE 8—*Concluded*

| RAT | | LITTER | | GESTATION | LACTATION | | |
|---|---|---|---|---|---|---|---|
| | | | | | Young weaned | | |
| Number | Age at mating | Number | Young | Oestrus on ? day | Number | Age | Oestrus ? days after delivery |
| E3 | 160 | 1 | 11 | 2, 5, 8, 11, 16 | 11 | 21 | B, 3, 5, 11, 13, 21, 24, 28 (C) |
| E3 | 209 | 2 | 10 | 2, 5, 9, 12, 16 | 9 | 30 | 1, 9, 16, 20, 22, 25, 27, 31 |
| E4 | 160 | 1 | 9 | 2, 8, 14, 17 | 9 | 21 | 5, 10, 18, 24 (C) |
| E4 | 218 | 2 | E | 15 | E | | B, 5, 8, 12, 17, 20, 25, 29 |
| E5 | 173 | 1 | 6 | 4, 7, 12 | 6 | 19 | B, 5, 11, 16, 20, 23, (C) |
| E5 | 218 | 2 | 7 | 2, 6, 9, 13, 16, 19 | 7 | 30 | 4, 10, 14, 19, 24, 27, 32, 36, 40 |
| F1 | 166 | 1 | 8 | 4, 13, 16 | 8 | 20 | 5, 12, 18, 22, 26 (C) |
| F1 | 214 | 2 | 3 | | E | | B, 5, 9, 13, 17, 21, 25, 29 |
| F2 | 153 | 1 | 9 | 4, 9, 13, 17 | 9 | 20 | 3, 6, 26 (C) |
| F2 | 200 | 2 | 3 | 5, 10, 14, 17 | 2 | 30 | 5, 10, 15, 26, 30, 34, 38, 42 |
| F3 | 87 | 1 | E | 8, 11, 15, 18 | E | | 3, 8, 14, 17, 19, 24, 28, 32 (C) |
| F3 | 142 | 2 | 11 | 2, 7, 11, 13 | 11 | 22 | B, 5, 7, 12, 20, 22, 26, 30, 34, 38, 42 (C) |
| F3 | 205 | 3 | 7 | 5, 9, 12 | 7 | 30 | B, 10, 14, 18, 24, 27, 34, 40, 44, 48 |
| F4 | 109 | 1 | 5 | 7 | 5 | 27 | 23, 27 (C) |
| F4 | 159 | 2 | 7 | 3, 5, 8, 11, 16 | 7 | 21 | B, 9, 13, 19, 24 (C) |
| F4 | 221 | 3 | 4 | 3, 7, 11, 13, 21 | 3 | 30 | 6, 18, 28, 32, 36, 40, 44 |
| F5 | 93 | 1 | 4 | 5, 9, 13, 17 | 4 | 30 | 2, 6, 13, 18, 25, 29, 33 (C 37) |
| F5 | 149 | 2 | 5 | 6, 13, 17, 20 | 5 | 21 | 15, 19, 24 (C) |
| F5 | 199 | 3 | 6 | 6 | 6 | 31 | 7, 11, 19, 23, 27, 29?, 31, 35, 39 |

lished following pseudopregnancy, and to the time at which pregnant mothers have died from overwork, we feel that this is possibly a critical time when abortion or resorption following copulation and fertilization may occur.

Whether the occurrence of oestruation during gestation is more prevalent at one age than another, we are unable to state at this time. The ages at mating of our rats (table 8) ranged from 87 to 288 days. From this data nothing definite can be stated relative to the effect of age. Experiments now in progress may throw some light on this as well as to the frequency that superfetation may occur.

The conclusions which we may draw are that though there is a great reduction in voluntary activity during gestation many spurts of activity resembling these exhibited by the non-pregnant animal during oestrus do occur; that these increases of activity tend to a four-day arrangement; and that the greatest grouping occurs about the twelfth or thirteenth day which time corresponds closely with the reëstablishment of oestrual periods in cases of pseudopregnancy.

*Oestruation during lactation.* In general we found that during approximately the first eighteen or twenty days of lactation oestruation, as shown

by a typical peak in the activity curve, was suppressed. Many peaks occurred but they were low in height. If weaning occurred about the twentieth day oestrual cycles, if not already noticed, were reëstablished within a few days. This is illustrated in figures 6 and 7A. These figures

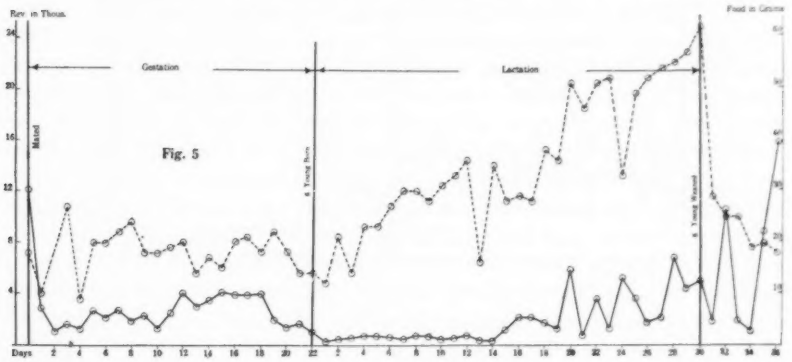


Fig. 5. Graph of rat $C_2$ from the age of 257 to 315 days, showing its activity (solid line) and food consumption (broken line) during its first gestation and lactation period of 30 days. This shows absence of oestruation during gestation and the first 15 days of lactation. The first oestruum occurred on the 16th day and continued at regular four-day periods thereafter even though six young were being nursed.

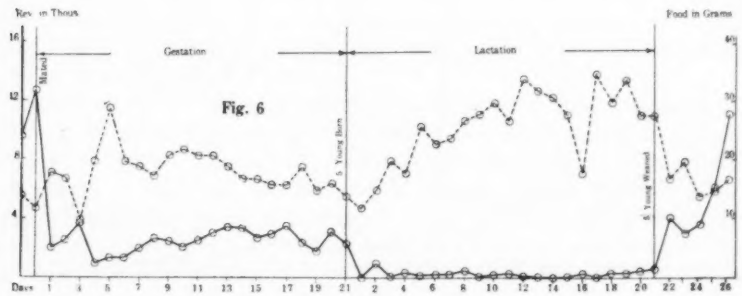


Fig. 6. Graph of rat $C_1$ from the age of 254 to 302 days showing its activity (solid line) and food consumption (broken line) during its third gestation period and lactation for 21 days. A typical oestrual peak is wanting until the 22nd day after birth, or the first day after weaning the five young.

show not only individual characteristics relative to the voluntary activity of these females, but also show that the food consumption varies directly with the number of young nursed. It is also seen that there were no cycles exhibited by these rats until after or on the day the young were weaned. The first oestruation after birth occurred in figure 6 on the

twenty-second day and in figure 7A on the twenty-first day (when she was remated—see fig. 7B). It is not an uncommon occurrence and we have had many instances where pairs kept in stationary cages have mated on

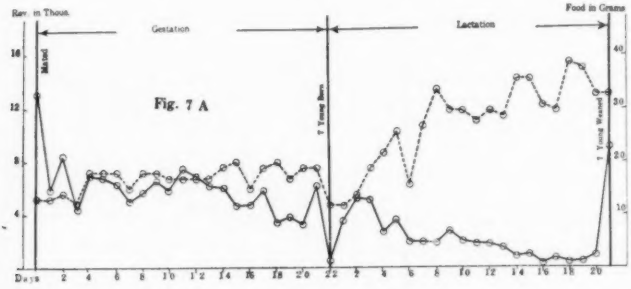


Fig. 7 A

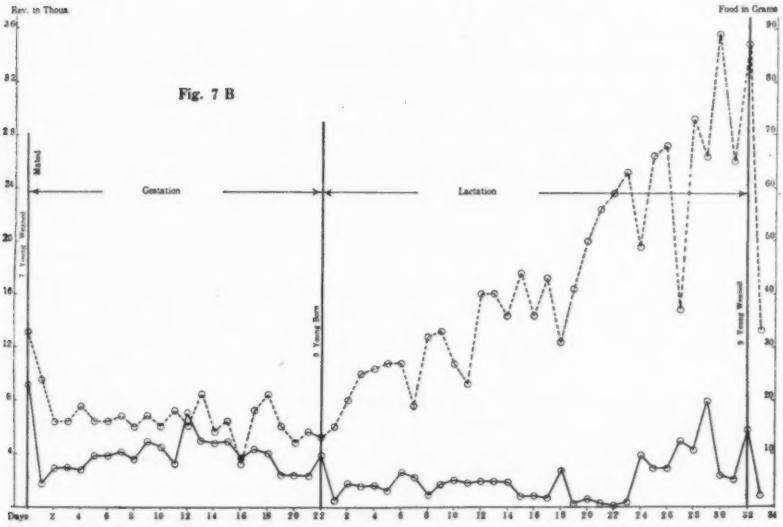


Fig. 7 B

Fig. 7 A. Graph of rat $D_2$ from the age of 153 to 194 days showing the activity (solid line) and food consumption (broken line) during its first gestation period and nursing seven young for 20 days. Absence of oestruation is noted.

B. Graph of rat $D_2$ continued from the age of 194 to 251 days showing the activity and food consumption during its second period of gestation and nursing of 9 young. Mating occurred on the 21st day of former lactation. Oestruation appears to have taken place on the 12th and 22nd day of gestation and on the 18th, 24th, 29th and 32nd day of lactation.

the day of birth of a litter and delivery of a second litter resulted in due time even though nursing the first litter. In such cases we have found

the second gestation period prolonged to as much as twenty-four days. Many cases of increased activity at birth suggest oestruation as having occurred at that time.

Examination of the data given in table 8 shows that there were many peaks exhibited by different rats in the activity curve during the first twenty days of gestation. Though these peaks of activity lack magnitude, when compared with those of the non-pregnant female, mating tests made at some of them have proved that they represent oestrus. During the first twenty days of lactation 160 peaks were observed. In fifteen of the cases (B) peaks of activity occurred on the day of birth of the litter. This indicates that about 27 per cent of the rats would have mated on that day. Forty-five had peaks prior to the twentieth day of lactation. In general these peaks were irregular in sequence and lacked the typical oestrual rhythm. Some showed but one peak with a long gap until a regular rhythm was established at about twenty days while others showed several at irregular intervals. In very few cases was a regular four-day rhythm established during the earlier days of lactation. When the data of the first twenty days are arranged in accordance with the day after birth on which the spurts of activity occurred, we find that though each day is represented they occurred on certain days more frequently than on others. The days which stand out prominently in this respect are: the day of delivery, the fifth, eleventh to fourteenth, and the eighteenth. The greatest number of peaks happened on the first and last of these days. This is significant and shows that there is a greater tendency on the part of the rat during the first twenty days of lactation to reëstablish oestruation on these days than on others.

In order to show the effect of prolonged lactation several females were allowed to nurse their young for periods varying from twenty-nine to thirty-four days. The activity and food consumption of a few of these are shown in figures 5, 7B, 8A and B. These figures illustrate that in general continued lactation beyond the twentieth day had little effect in retarding the onset of the oestrual cycles. They were usually established in nursing mothers about the twentieth day of lactation. The number of young nursed did not appear to influence the reëstablishment of oestrus. Individual variation is quite marked. For example, the activity of rat B3 indicated that oestruation was established in fairly definite rhythm throughout the entire nursing period.

From these observations we may conclude that in many cases oestrual cycles are wanting during the first twenty days of lactation and that they are reëstablished about this time irrespective of the length of the lactation period or of the number of young nursed. Exceptions are sufficiently numerous to enable us to substantiate the investigations of others, both as to the occurrence and absence of oestruation during at least the first

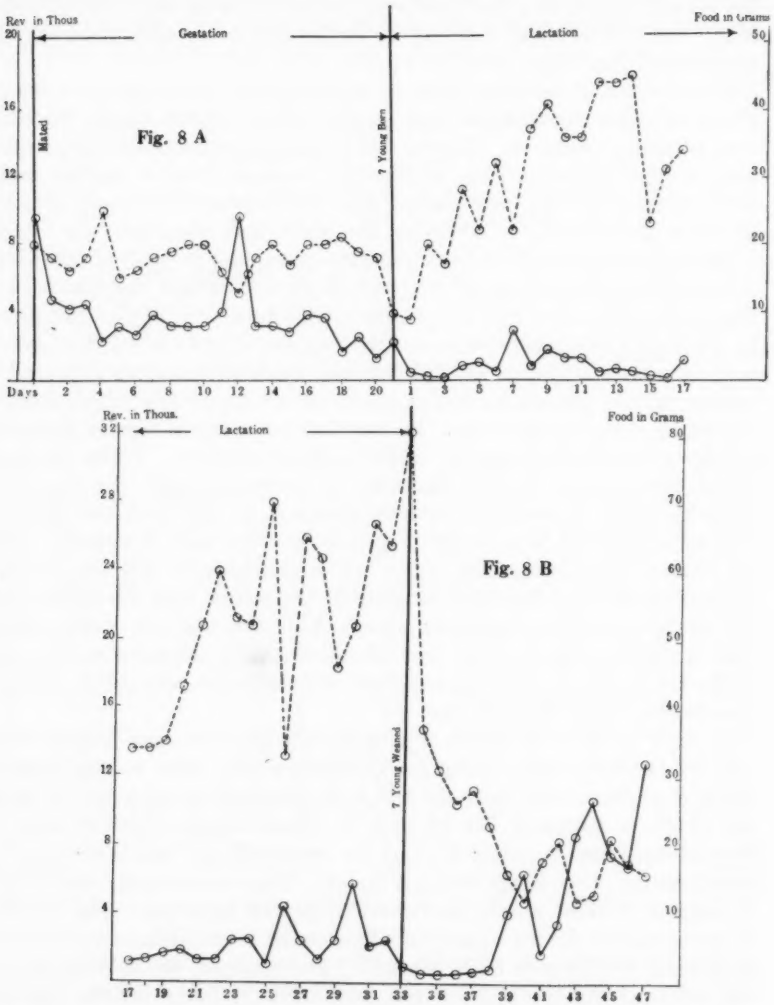


Fig. 8 A. Graph of rat $D_1$ from the age of 192 to 230 days, showing the activity (solid line) and food consumption (broken line) during its second gestation and the first 17 days of lactation, 7 young being nursed. This shows a typical oestrual peak on the 7th day of lactation.

B. Continuation of graph of rat $D_1$ during the last part of the period of lactation. Typical oestrual peaks are shown on the 26th and 30th days of lactation followed by a suppression until the 40th day after delivery.

twenty days of lactation. We may further conclude that there is a greater tendency on the part of the rat to reëstablish oestruation on certain days of early lactation than on others.

*Effect of eating the young.* Cannibalism in the albino rat is not uncommon. Of the fifty-six litters born in this experiment, ten were eaten by the mother either at birth or within a few days after delivery. By consulting table 8 it will be seen that there is no case where one mother ate more than one of her litters. It is also noticed that the litters eaten were either the first or the second, there being five of each. With the exception of B1, litter 2 and C1, litter 1, the first oestrus after delivery occurred within a few days. In the two exceptions a regular oestrual rhythm was established on the thirteenth and fourteenth days after delivery. Whether the early establishing of oestrus in the majority of these mothers was due to the cessation of lactation or to the enriched animal protein diet we are unable to state, but it is very probable that each exerted a decided influence. The curves of activity and food consumption of these rats conform to the typical manner of reëstablishing oestrual rhythm following cessation or partial suspension due to mating, gestation, parturition or lactation. That is, the first peak is low, the second higher, the third still higher, and so on until they reach the normal height for the age of the individual. In other words, the curve of voluntary activity during reëstablishment of oestrual rhythm corresponds very closely to that of the establishment of the rhythm in the young animal following pubescence.

As would be expected, the consumption of the synthetic food given was less during the first few days following parturition while the young were being eaten than it was with mothers which nursed their young. The average weight of the food given that was eaten by mothers which had eaten their young for each of the first six days following parturition was 11, 13, 13, 13.5, 13 and 15 grams. The average amounts consumed by nursing mothers for the same days was 14.4, 16.6, 19.5, 21.4, 24 and 25.4 grams.

These results show that the eating of the young, followed by the discontinuance of lactation, is favorable to the reëstablishment of the oestrual rhythm at an early date after parturition.

*Removal of young at birth.* A number of tests have been made to show the effect of removal of the young at birth on the reëstablishment of oestrus. This was done to further show that lactation and nursing tended to suppress oestruation. In these tests females were paired and the pair isolated in stationary cages. As soon as the females showed signs of pregnancy the males were removed. On the day of delivery the young were removed and destroyed and the males returned. In this way copulation and fertilization presumably occurred at the first oestrus after

delivery. A number of successive litters of the same individuals have been followed in this way. The results are given in table 9. This table shows that the first oestrus after delivery occurred at varying times ranging from the day of birth of the litter to as late as the twenty-second day after birth. It also shows that much individual variation occurred. Seventeen of these twenty-one cases happened on or before the eighth day, and ten by the fourth day. The error in these observations, if any existed, was in favor of earlier oestrus for we have found many cases in which copulation did not result in successful fertilization, or in a delay of succeeding oestrual cycles. The longer delay of reëstablishment of oestrus in some of the mothers which ate their young or had their young removed at birth may have been due to a disturbance or illness induced by the abrupt cessation of nursing and congestion of the mammary glands.

TABLE 9

*Showing the age of the mother at birth of each litter, the number of young born, and the day after delivery at which oestrus and fertilization of the next litter occurred. We have assumed the average gestation period of twenty-two days in all cases*

| RAT | LITTER 1 | | | LITTER 2 | | | LITTER 3 | | | LITTER 4 | | | LITTER 5 | | | LITTER 6 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Age | Number of young | Day mated | Age | Number of young | Day mated | Age | Number of young | Day mated | Age | Number of young | Day mated | Age | Number of young | Day mated | Age | Number of young | Day mated |
| 1 | 112 | 7 | 8 | 142 | 7 | 12 | 176 | 8 | 3 | 201 | 9 | 6 | 230 | 10 | 1 | 253 | 5 | |
| 2 | 102 | 3 | 22 | 146 | 5 | 0 | 168 | 8 | 1 | 191 | 8 | 2 | 215 | 10 | 12 | 249 | 4 | |
| 53 | 120 | 8 | 11 | 153 | 7 | 6 | 181 | 8 | 4 | 207 | 9 | 7 | 236 | 11 | | | | |
| 54 | 120 | 11 | 7 | 149 | 9 | 4 | 175 | 8 | 4 | 201 | 10 | 5 | 228 | 8 | 8 | 258 | 6 | |
| 2D-2 | 154 | 7 | 1 | | | | | | | | | | | | | | | |
| 2D-5 | 134 | 12 | 5 | | | | | | | | | | | | | | | |

These data seem to indicate that the removal of the young at birth has little, if any, more favorable influence on the reëstablishment of the oestrual rhythm following parturition than the eating of the young by the mother.

*Copulation, stimulation of cervix uteri, and pseudopregnancy.* Following a successful copulation there was almost always a conspicuous and pronounced drop in the voluntary activity during the succeeding twenty-four hours. The average number of revolutions turned by our female rats on the day that they were mated was 16,352. The average for the following day was 2,779 revolutions. Great variations in the extent of the drop in activity has been observed in different individuals. The difference between the high point at mating and the low point on the succeeding day ranged from a few thousand to as many as thirty or forty thousand revolu-

tions. The great variation was in the high point and not in the low point. Following the low point there was usually a slight and gradual increase in activity as previously described for gestation. Exceptions to this characteristic drop have been noted. This drop was always characteristic of copulation and could usually be relied upon as indicating insemination. The cause of the reduction of activity was apparently the result of coitus or, possibly, of excessive activity connected therewith producing extreme fatigue. That the latter could be only a contributing factor was shown by the fact that we have had many successful matings which resulted from a single coitus and in which the two sexes were together but a few minutes. During this short time very little activity was exhibited by the female during copulation and yet the characteristic drop in activity occurred. From this it would appear that though fatigue may have had something to do with the drop in activity following an oestrual peak, it was not sufficient to always cause the pronounced drop following coitus. Rat W3 showed that the drop to the lowest point in dioestrum occurred during various intervals of time ranging from one to three days, and that the greatest drop did not necessarily follow the highest peak in activity. Since it is conceded that the oestrual peaks are due to the activation of the follicular fluid hormone, the drop in activity following oestruation must be due partly, at least, to the using up of this hormone or to its inhibition. Coitus apparently causes this hormone to be depleted more rapidly, or in some way produces a more rapid and lasting inhibition of its action. That this is not due to a chemical stimulus caused by the presence of the seminal fluid is shown by the fact (as shown by others) that artificial stimulation of the cervix uteri with a glass rod while the animal was in heat caused a drop in activity similar to that following coitus. Just how this is brought about we are unable to state at this time. Long and Evans think that the vaginal plug plays an important part, but this does not explain the results following artificial stimulation of the cervix uteri. It is generally thought that the function of the corpora lutea is to hold in check the next oestrual cycle and thus permit implantation if the ova have been fertilized. The corpora lutea therefore may possibly exert a sudden inhibiting influence on voluntary activity. If the ova were not fertilized the influence would last only the few days of dioestrum; but if they were fertilized it would last during the period of gestation and lactation if the young were nursed. Long and Evans state that during the first nine or ten days of gestation the corpora lutea gravidatatis closely resemble the corpora lutea ovulationis in size and in number, and size and distribution of the lipoid granules. After the tenth day the corpora lutea of gestation increase in size mainly by growth of the individual lutein cells. This final increase in size does not occur in the corpora of ovulation which gradually decrease and disappear. The persistence of the corpora lutea of gestation

and lactation and the small amount of voluntary activity exhibited by the animal during this time very strongly suggest a relation of cause and effect. We are unable to give any suggestions as to how, in many cases, later oestrual cycles are able to break through this apparent inhibitory influence at various times in gestation and lactation during which another mating and fertilization may occur.

We have had a number of cases of mating where copulation not only appeared successful, but in which vaginal examination showed that insemination had occurred, that did not result in full term gestation and

TABLE 10

*Cases of pseudopregnancy. Showing the age of the female at mating and the number of succeeding days intervening before the first oestrus*

| FEMALE | | DAYS AFTER MATING OF FIRST OESTRUS |
|---|---|---|
| Number | Age at mating | |
| B 3 | 110 | 15 |
| B 3 | 136 | 15 |
| C 1 | 129 | 15 |
| C 2 | 122 | 11 |
| C 5 | 126 | 14 |
| D 3 | 80 | 13 |
| D 3 | 117 | 15 |
| D 4 | 115 | 15 |
| D 1 | 135 | 16 |
| D 5 | 89 | 12 |
| W 1 | 197 | 15 |
| W 2 | 225 | 14 |
| W 3 | 250 | 15 |
| W 3 | 290 | 14 |
| W 4 | 255 | 13 |
| W 4 | 275 | 14 |
| W 5 | 282 | 16 |
| W 6 | 255 | 15 |
| W 7 | 255 | 14 |
| W 8 | 199 | 15 |
| Average..... | 182 | 14.3 |

delivery of young. The curve of voluntary activity in all cases was characteristic of gestation for about the first fifteen days following coitus. The data of incomplete gestations are given in table 10. This table shows the age of the female at mating and the number of days later at which the oestrual cycles again occurred. That is, there was a cessation of oestruation for that number of days following coitus, during which time the activity curve was typical of gestation. About fourteen or fifteen days (eleven to sixteen in our cases) after coitus, typical oestrual cycles were

reëstablished. This phenomenon has been designated pseudopregnancy by other writers. This table shows that no correlation can be made between the age of the rat and the duration of pseudopregnancy. As we have mentioned above, it is about this time during gestation that an oestrus is likely to occur, and that this appears to be a critical time when gestation may be disturbed causing resorption, abortion, and possible death of the mother due to oestrus and coitus. The work of Long and Evans seems to substantiate this. They found that the vaginal speculum disclosed "a bright red, bloody discoloration of the floor of the vagina, especially in its upper part near the cervix, a discoloration actually due to the presence of free blood upon the surface of the mucosa." This condition was found about the fifteenth day of gestation and lasted approximately three days. They think it was probably due to "a leakage of blood of uterine, and, presumably, placental origin, which has escaped through the cervical canal." They consider this the earliest infallible sign of pregnancy in the living animal.

We have now no means of determining whether fertilization and resorption, or abortion, occurred in our cases of pseudopregnancy. The fact that pseudopregnancy can be induced (Long and Evans, and Wang) by stimulation of the cervix uteri with a glass rod proves that fertilization is not necessary to produce the phenomenon. It thus appears to be due to a mechanical stimulus which in some way, probably through the nervous system, causes the corpora lutea of ovulation to maintain their inhibiting influence on succeeding oestrual cycles for this length of time.

In some cases we have had the typical effect of coitus followed by the reëstablishing of oestrus within a few days. This was illustrated by rat W8. The first oestrus appeared on the seventh day following copulation. This peak, which was low, was followed each fourth day by peaks gradually increasing in height until they reached the maximum. In this case, in which only one oestrual cycle was suspended, the reëstablishing of oestrual rhythm was very similar to that described following lactation, and to the original establishing of the rhythm at puberty.

In many cases we have had considerable difficulty in getting the animals to mate after they had passed through a period of pseudopregnancy. Rat W8 illustrates one of these cases. This female was placed with a strong and vigorous male at different peaks of her activity and the number of copulatory acts in each case noted. Though copulation took place in several trials pregnancy and delivery did not follow. Not infrequently has coitus, following pseudopregnancy, resulted in another pseudopregnancy. The frequency of these results in different individuals indicates abortion, resorption, or a possible injury of some sort.

That injury may result from artificial stimulation of the cervix uteri and may be a cause of pseudopregnancy is indicated by the work of Long

and Evans. They state that pseudopregnancy results from artificial stimulation only when the stimulus is applied during stages one to three of the oestrual cycle, that is, from shortly before to shortly after the period of heat. If we interpret their results correctly, out of ten trials at other times in the cycle they got pseudopregnancy in 50 per cent of the cases. In these instances the rod used may have produced injury which caused the delay in succeeding cycles. In over one hundred other cases, in which the stimulus was given at the stipulated time, only 66 per cent resulted in pseudopregnancy. Wang also found that only a certain per cent of the trials proved successful. We have found that when the glass rod was used with great care none of the tests resulted in pseudopregnancy. In no case have we induced pseudopregnancy when the stimulus was carefully given at other times than near the oestrual peak of activity.

In experiments now in progress to show the effect of early and late breeding during oestrus we have found that in late breeding pseudopregnancy resulted in eighteen out of nineteen tests in which vaginal smears showed the presence of sperm. This again is rather suggestive. When females are bred late in heat they do not accept the male readily, but tend to fight and resist. Coitus under such conditions would be more likely to result in injury to the female than when the male is willingly accepted. The presence in the vaginal smear of numerous erythrocytes following coitus indicates injury. Later work may throw further light on the cause of this phenomenon.

These results and those referred to show that following successful copulation and successful stimulation of the cervix uteri there was a decided and characteristic drop in the voluntary activity of the female similar to that preceding pregnancy; that in many cases, especially of coitus late in heat and of artificial stimulation, there resulted a condition called pseudopregnancy which lasted about fifteen days during which time the curves of activity and food consumption resembled those of true pregnancy; that pseudopregnancy may be due to a nervous stimulus, injury, abortion or resorption; that the regular oestrual rhythm is reëstablished about the fifteenth day; and that the reëstablishment of oestrual rhythm is similar to its first establishment at puberty or its reëstablishment after lactation.

## SUMMARY

From the above discussion the following conclusions may be summarized:

1. Sexually mature non-pregnant females showed regular rhythm of activity and food consumption. The low point in food consumption occurred during the peak of activity.

2. The average daily activity and food consumption of non-pregnant females were about 9000 revolutions and 18 grams. The average daily activity and food consumption of pregnant rats were 3388 revolutions and 20 grams.

3. The average daily activity and food consumption decreased during the last days of gestation. This was especially noticeable the last day of gestation.

4. There was a characteristic drop in average activity following successful coitus. The average activity for the twenty-four hours preceding mating was 16,352 revolutions; for the twenty-four hours following mating it was 2,779 revolutions. This drop can usually be relied upon as indicating successful mating.

5. During lactation the average daily activity was less than during pregnancy, but the average food intake increased in proportion to the growth and to the number of young that were nursed.

6. Mothers carrying large litters were more active and consumed less food during gestation than mothers carrying small litters.

7. Mothers with large litters had a shorter gestation period than mothers with small litters. This was more pronounced in young animals than in old ones.

8. Mothers nursing large litters were more active and consumed more food during lactation than those with small litters.

9. The amount of food consumed depended on the number of young nursed, the activity of the mother, and on her age; that is, whether she was a mature or a growing animal.

10. Typical oestrual rhythm was absent during gestation and the first twenty days of lactation.

11. Many cases in which one or more oestrual cycles, occurring during both gestation and lactation, were observed.

12. Oestrus during gestation was found more frequently about the fourteenth day than at any other time.

13. In cases of pseudopregnancy oestruation was usually reëstablished about the fourteenth or fifteenth day following coitus.

14. Coitus late in heat resulted in pseudopregnancy in about 90 per cent of the trials.

15. We have been unsuccessful in inducing pseudopregnancy by careful artificial stimulation.

16. Results indicate strongly that pseudopregnancy was caused by some injury.

17. Oestruation was usually reëstablished about the twentieth day of lactation. The length of the lactation period or the number of young nursed did not appear to have any marked influence on the reëstablishing of oestruation.

18. The reëstablishing of oestrual rhythm was similar to the beginning of the rhythm at puberty.

19. Eating of the young, or removal of them at birth, caused an earlier reëstablishing of oestrual rhythm. The average of thirty-one cases was 5.6 days after parturition.

20. Various results indicate that about the fourteenth day of gestation is a critical time in the rat when abortion, resorption, oestrus, and possibly death are likely to occur.

21. Owing to the wide variation exhibited by different individuals we have been able to verify the diverse results of other investigators

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# BLOOD VOLUME CHANGES AT HIGH ALTITUDE

H. P. SMITH, A. E. BELT, H. R. ARNOLD AND E. B. CARRIER

*From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco*[1]

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This paper is particularly concerned with a study of both blood plasma and red cell volumes as influenced by residence at high altitude. It is now generally accepted that the red cell count and hemoglobin values increase definitely during a stay of a few weeks at high altitude but there are not accurate data available in the literature dealing with the influence of altitude upon the plasma and red cell volumes. The tables below show that the blood plasma volume is little disturbed while the red cell and hemoglobin volumes are definitely increased by residence of four weeks at high altitude.

Accepting as established facts the increase in red cell counts and hemoglobin values at high altitude, one may choose to explain this reaction as due to shrinkage of the plasma volume, to redistribution of cells lying inactive in the marrow, spleen or other sinuses, or to a true increase of red cell and hemoglobin volumes. Our experiments indicate that the last explanation is correct.

One of the obstacles in the way of obtaining dependable total blood volume figures has been the habit of assuming that the blood cells and plasma are uniformly mixed in all parts of the circulating system. Thus in the method where the *volume of blood plasma* is to be estimated a known amount of brilliant vital red is injected into the circulating blood. After complete admixture the concentration of the injected substance in the blood plasma is determined. The volume of plasma required to dilute the total mass of injected dye is then computed. A figure for the *plasma volume only* is thus obtained. If reliance is placed upon this figure alone and no attempt is made to compute the total blood volume from the ratio of cells and plasma in a given sample of circulating blood the error due to unequal distribution of cells will be avoided.

In a similar manner the actual *volume of red blood cells* may be estimated. A known volume of carbon monoxide is exposed for absorption by the blood which circulates through the lungs and the amount of ab-

[1] Aided by a grant from the University of Rochester School of Medicine and Dentistry. G. H. Whipple took part in the preliminary work but not in the expedition.

sorbed gas in a measured sample of packed red cells is determined. From the degree of dilution of the gas in the specimen of packed red cells the total body hemoglobin is estimated. By using both methods simultaneously on the same individual the value of cell and plasma volume are each independently determined.

A critical review and study of the methods and problems related to blood plasma volume, red cell and hemoglobin volumes has recently appeared from this laboratory (1), (4), (7). These various methods, controls, etc., are carefully reviewed in these papers and the methods were thoroughly familiar to all the research members of this expedition. This expedition was planned to test out various theories about blood volume and hemoglobin volumes as influenced by residence at high altitude. It seemed an unusual opportunity to test these various altitude reactions of the circulatory system using the most modern and accurate methods.

EXPERIMENTAL CONDITIONS. The experiments reported here were begun in the month of June, 1921. Preliminary to the studies at high altitude a number of normal controls were done in the laboratory at San Francisco. The experiments at high altitude were carried out in the Sierra Nevada Mountains at Long Lake a few miles distant from Bishop, California. The trip from San Francisco to Bishop (altitude 4000 feet) was made partly by train, partly by automobile. From Bishop the trip to Andrew's camp (altitude 8000 feet) was made by automobile. From here on the trip to Long Lake (altitude 11,000 feet) was made partly on foot but largely on horseback. In all the trip from San Francisco to Long Lake took four days. Camp was then promptly established. A day or two later blood volume determinations were begun.

From this account it is clear that the members were not entirely exempt from exertion in making the trip and in establishing camp. Such effort cannot be wholly disregarded from the experimental viewpoint. It may be noted, however, that the daily exercise was not much more rigorous than that involved in the preparatory control period at sea level. The time spent at Long Lake varied somewhat for different members of the party but all remained approximately four weeks. During this period all members were busy about camp, either with experimental work or with general camp duties. On several occasions brief trips were made into the surrounding mountains. From Long Lake the party returned to San Francisco. Here control experiments were again carried out over a number of weeks as indicated in the individual tables.

The temperature variations at Long Lake were somewhat greater than at San Francisco. When the party first arrived at Long Lake there was snow in the immediate vicinity of the camp site. Later on snow was found only on the distant mountain sides or in the nearby ravines. The nights were cool (2° to 3°C.) but the days were warm (20° to 30°C.).

The sunlight was considerably more intense than at San Francisco. The camp was well protected from wind and was situated just at the tree line so that plenty of wood was available, while Bishop Creek, fed largely by the snow fields in the vicinity of Bishop Pass about a mile upstream, provided water. An abundant variety of fresh food was packed into camp twice a week. Barometric pressure varied between 505 and 513 mm. mercury.

EXPERIMENTAL TECHNIC. The experimental technic was uniform throughout the course of the experiments. *Blood counts* were done with the Thoma-Zeiss counting chamber. Blood for the counts was taken from a needle inserted into the basilic vein in the hollow of the elbow. Comparisons of this venous blood with blood taken from the ordinary skin puncture were made on a number of occasions but no difference could be demonstrated. Duplicate pipettes were always taken and preparations made from each pipette were counted. In each case at least three different preparations were examined and if the differences between extremes was over 400,000 red cells further counts were made until such variations could be minimized by averaging the results. We feel that errors in counting the blood rarely exceed 200,000 cells per cubic centimeter.

*Hemoglobin estimations* were carried out with the duBoscq colorimeter and the Newcomer standard glass (5). Daylight was used for making the readings. A number of hematin solutions prepared for examination at Long Lake were carried back to San Francisco and again read against the standard. The readings obtained were the same as those in the mountains which shows that no error arose from differences in the quality of daylight at the two places.

*Hematocrit determinations.* Centrifugalization of blood was carried out at Long Lake with the aid of a Daland hand centrifuge capable of carrying either 15 cc. tubes or graduated micro tubes. The latter were used for all hematocrit determinations. The rate was 3500 revolutions per minute. Readings were taken at the end of 20, 30 and 60 minutes. In almost all cases packing was practically complete at the end of 30 minutes. In the experiments at sea level a large laboratory centrifuge carrying 15 cc. hematocrit tubes was used in most of the experiments. A rate of 3000 revolutions a minute was maintained. Using the same blood a comparison of the two centrifuges showed that with the micro tubes and the Daland centrifuge the packing was about 1.5 per cent more complete than with the larger tubes. Because of this discrepancy the sea level figures obtained with the large centrifuge were corrected to agree with the readings obtained on the Daland centrifuge.

*Blood volume methods.* Instead of employing only one blood volume method we used two, the dye and the CO methods jointly. The ad-

vantages of combining these two procedures have been discussed at length in earlier papers from this laboratory (7), (4), (1). It will suffice to recall here that dye injected into the blood stream mixes with the plasma.

TABLE 1

*H. R. A.*

| DATE | R.B.C. | HEMOGLOBIN PER 100 cc. BLOOD | HEMATOCRIT (RED CELLS) | CARBON MONOXIDE METHOD | | | | DYE METHOD | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Red cell volume | Blood volume | Plasma volume | Total body hemoglobin | Plasma volume | Blood volume | Red cell volume |
| *1921* | *thousands* | *gms.* | *per cent* | *cc.* | *cc.* | *cc.* | *gms.* | *cc.* | *cc.* | *cc.* |
| Sea level | | | | | | | | | | |
| 6/3 | 5158 | | 45.4 | 1945 | 4284 | 2340 | | | | |
| 6/4 | | | 45.3 | | | | | 3432 | 6273 | 2841 |
| 6/8 | 4918 | 15.2 | 43.4 | 1990 | 4585 | 2595 | 697 | | | |
| 6/9 | | | 43.1 | | | | | 3648 | 6408 | 2760 |
| 6/14 | | | 42.2 | | | | | 3402 | 5892 | 2490 |
| 6/15 | 5168 | 13.6 | 42.3 | 2070 | 4894 | 2825 | 666 | | | |
| Average | 5081 | 14.4 | 43.6 | 2002 | 4588 | 2587 | 682 | 3494 | 6191 | 2697 |
| High altitude | | | | | | | | | | |
| 6/25 | 5108 | 15.1 | 42 6 | 1930 | 4530 | 2600 | 684 | | | |
| 6/26 | | | 42.9 | | | | | 3433 | 6008 | 2575 |
| 7/5 | 5437 | 16.1 | 45.2 | 1978 | 4376 | 2400 | 705 | | | |
| 7/15 | 5369 | 17.2 | 45.3 | 2160 | 4768 | 2610 | 820 | | | |
| 7/19 | | | 45.0 | | | | | 3405 | 6190 | 2785 |
| 7/21 | 5871 | 17.5 | 49.0 | 2295 | 4684 | 2390 | 820 | | | |
| Sea level | | | | | | | | | | |
| 7/31 | 5304 | 17.5 | 45.2 | 2120 | 4690 | 2570 | 821 | | | |
| 8/11 | 5369 | 16 4 | 43.9 | 2020 | 4601 | 2581 | 755 | | | |

Male, Weight 62 kgm.

June 18: Left San Francisco. Arrived at Long Lake on June 21. Considerable shortness of breath on first reaching high altitude, only upon exertion. There was no cyanosis. For the first few nights he was occasionally awakened by feeling of air hunger. Some periodicity in breathing was noted at times. Headache at times, during the first few days, particularly in the early morning. Shortness of breath much less marked after about a week. Approximately 30 minutes following the dye injection on June 20 a violent headache developed. No disturbance in pulse. Given phenacetin. Headache much better after two hours. There was relatively little headache following administration of carbon monoxide and with the exception of the experiment on June 26 there was never any severe reaction from the injection of brilliant vital red.

June 25 to 27: Return trip to San Francisco.

From the concentration of dye in the plasma drawn for analysis the total *volume of plasma* is determined. From this figure and the hematocrit

the cell volume and total volume may be determined indirectly. The carbon monoxide method differs from this in that the CO is taken up from the respiratory chamber into the lungs and thence into the blood

TABLE 2

*A. E. B.*

| DATE | R.B.C. | HEMOGLOBIN PER 100 CC. BLOOD | HEMATOCRIT (RED CELLS) | CARBON MONOXIDE METHOD | | | | DYE METHOD | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Red cell volume | Blood volume | Plasma volume | Total body hemoglobin | Plasma volume | Blood volume | Red cell volume |
| *1921* | *thousands* | *gms.* | *per cent* | *cc.* | *cc.* | *cc.* | *gms.* | *cc.* | *cc.* | *cc.* |
| Sea level | | | | | | | | | | |
| 6/2 | | | 44.7 | | | | | 3435 | 6210 | 2775 |
| 6/4 | 5425 | 16.1 | 48.5 | 2033 | 4192 | 2159 | 675 | | | |
| 6/9 | | | 45.7 | | | | | 3400 | 6265 | 2865 |
| 6/10 | 5350 | 15.8 | 46.6 | 2190 | 4700 | 2510 | 743 | | | |
| Average | 5388 | 16.0 | 46.4 | 2112 | 4446 | 2335 | 709 | 3418 | 6238 | 2820 |
| High altitude | | | | | | | | | | |
| 6/26 | 5500 | 16.4 | 48.2 | 2260 | 4689 | 2429 | 769 | | | |
| 7/6 | 6538 | | | | | | | | | |
| 7/8 | 6516 | 17.9 | 50.2 | 2645 | 5269 | 2624 | 943 | | | |
| 7/16 | 6549 | 17.9 | 49.9 | 2530 | 5090 | 2560 | 911 | | | |
| 7/18 | 6261 | | 46.0 | | | | | 3615 | 6695 | 3080 |
| 7/21 | 6422 | 17 9 | 51.0 | 2850 | 5588 | 2738 | 1000 | | | |
| Sea level | | | | | | | | | | |
| 7/31 | 5573 | 17.5 | 51.5 | 2810 | 5456 | 2646 | 955 | | | |
| 8/13 | 5516 | 17.2 | 48.6 | 2770 | 5700 | 2930 | 980 | | | |
| *1922* | | | | | | | | | | |
| 5/28 | 4928 | 14.3 | 43.2 | 2000 | 4630 | 2630 | 662 | | | |

Male, weight 71 kgm.

June 18: Left San Francisco and arrived at Long Lake June 21. Shortness of breath and dizziness only with considerable exertion. Occasional headaches, though not much more common or severe than at sea level. Cyanosis of lips and ears rather conspicuous throughout stay at Long Lake; however cyanosis is sometimes observed also at sea level, but never are any ill effects associated with it. This subject had severe headache and often chills following intravenous injection of brilliant vital red. The pulse and respiration were never affected. The symptoms were noted at sea level as well as in the mountains. Headaches also regularly followed the carbon monoxide blood experiments. These seemed to be somewhat exaggerated at high altitude.

July 25 to 27: Return trip to San Francisco. May 25, 1922, trip by train to Baltimore, Md. Blood volume determinations repeated at that place.

where it mixes not with the plasma but with the hemoglobin containing cells of the circulating blood. Hence the amount of CO extracted from a

sample of the blood is an index of the total *red cell volume* and the plasma and total volume can only be calculated indirectly by this method. When

TABLE 3

*H. P. S.*

| DATE | R.B.C. | HEMOGLOBIN PER 100 CC. BLOOD | HEMATOCRIT (RED CELLS) | CARBON MONOXIDE METHOD | | | | DYE METHOD | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Red cell volume | Blood volume | Plasma volume | Total body hemoglobin | Plasma volume | Blood volume | Red cell volume |
| *1921* | *thousands* | *gms.* | *per cent* | *cc.* | *cc.* | *cc.* | *gms.* | *cc.* | *cc.* | *cc.* |
| Sea level | | | | | | | | | | |
| 6/3 | | | 42.5 | | | | | 3810 | 6628 | 2818 |
| 6/4 | 4848 | 16.1 | 46.8 | 2185 | 4669 | 2484 | 752 | | | |
| 6/8 | 5293 | 15.8 | 47.0 | 2160 | 4596 | 2436 | 726 | | | |
| 6/9 | | | 45.7 | | | | | 3642 | 6707 | 3065 |
| Average | 5072 | 16.0 | 45.5 | 2173 | 4633 | 2460 | 739 | 3726 | 6668 | 2942 |
| High altitude | | | | | | | | | | |
| 6/27 | 5376 | 16.4 | 48.4 | 2285 | 4721 | 2436 | 774 | | | |
| 7/6 | 5678 | 17.9 | 52.6 | 2540 | 4829 | 2289 | 864 | | | |
| 7/16 | 5639 | 17.9 | 49.3 | 2565 | 5203 | 2638 | 931 | | | |
| 7/23 | 5642 | 17.2 | 47.6 | 2455 | 5158 | 2703 | 887 | | | |
| 7/24 | | | 43.9 | | | | | 3350 | 5975 | 2625 |
| 7/28 | 5750 | 16.4 | 50.0 | 2530 | 5060 | 2530 | 830 | | | |
| 8/2 | 5578 | 16.8 | 49.0 | 2438 | 4977 | 2539 | 836 | | | |
| Sea level | | | | | | | | | | |
| 8/10 | 5284 | 17.5 | 49.0 | 2590 | 5288 | 2698 | 925 | | | |
| 8/18 | 5160 | 17.2 | 45.3 | 2350 | 5188 | 2838 | 892 | | | |
| 9/18 | 4904 | 14.8 | 46.6 | 2205 | 4732 | 2527 | 700 | | | |
| 11/6 | 5140 | 15.4 | 47.5 | 2240 | 4716 | 2476 | 726 | | | |
| *1922* | | | | | | | | | | |
| 3/10 | 5253 | 16.4 | 44.9 | 2170 | 4833 | 2663 | 793 | | | |
| 5/26 | 5356 | | 46.0 | 2250 | 4891 | 2641 | | | | |
| 6/5 | 5014 | | 45.8 | 2090 | 4563 | 2473 | | | | |

Male, weight 71 kgm.

June 18: Left San Francisco and arrived at Long Lake on June 21. Noticeable shortness of breath on reaching high altitude. Orthopnea during the first 5 or 6 nights. Slight headache occasionally for a few days. Never any severe reactions from dye injection. Sometimes moderate headache following inhalation of carbon monoxide.

August 2 to 4: Return trip to San Francisco. August 20, left San Francisco for Baltimore, Md. The rest of the determinations were done in the Department of Pathology, Johns Hopkins Medical School.

both methods are used the plasma and cell volumes may each be determined directly.

The detailed technic follows: For the dye blood volume determinations a needle was inserted into the basilic vein at the bend of the elbow and a 10 cc. sample of blood removed for preparing the standard. At this time blood for the R.B.C. count and hemoglobin determinations was also taken. Ten centimeters of a sterile 1 per cent aqueous solution of brilliant vital red were then injected into the vein and repeatedly washed in with blood drawn back into the record syringe. The needle was removed. Four minutes later the needle was again inserted into a vein and another 10 cc. sample removed. Both samples of blood were mixed with 1 cc. of 1.6 per cent (isotonic) sodium oxalate solution. From these samples the hematocrit tubes were filled. The per cent R.B.C. was determined as described above. The details of diluting the dye-stained plasma and comparing it with the freshly made standard are identical with those given for dogs (4), and need not detain us here.

The carbon monoxide method was also carried out in a manner similar to that described for dogs (1). The respiratory chamber consisted of two metal cylinders closed at one end. They were 18 inches in height. The larger vessel was 12 inches in diameter and the smaller 10 inches. The larger vessel contained a solution of concentrated NaOH. Inside the smaller vessel a number of bars of metal were hung to increase the surface area. This vessel was inverted and set in the NaOH. A tube, soldered into the bottom, was connected with the supply of CO and the $O_2$ tank. Finally a pulley and weight was attached to the smaller of two cylinders. The weight was so adjusted that the cylinder moved up and down with a minimum of effort. The principle is the same as that used in certain types of vital capacity machines.

At the beginning of the experiment the cylinder was partly filled with nitrogen and about 30 per cent oxygen. The subject then breathed back and forth into it a few times and then the carbon monoxide was slowly admitted over a period of 2 minutes. As the alkali absorbed the $CO_2$ exhaled by the patient, fresh oxygen was supplied. Respiration could be carried on into this chamber with very little discomfort. In our experiments the subjects inhaled the CO mixture for 8 minutes. Two minutes later a needle was inserted into the basilic vein and about 6 to 8 cc. of blood withdrawn by syringe into a tube containing dry sodium oxalate. Blood for the counts, hematocrit and hemoglobin was taken at this time.

The procedure for the analysis of the blood was identical with that already reported except that one additional control was introduced.

After the gases had been liberated and extracted from the blood in the Van Slyke machine, and after absorption of the oxygen with pyrogallol, the amount of carbon monoxide present was determined directly by introducing a small amount of acid cuprous chloride which absorbed

the carbon monoxide and left only the nitrogen. In contrast to the old indirect method of making a constant deduction for nitrogen and assuming the rest to be carbon monoxide, this procedure gives a direct measure of the amount of carbon monoxide present.

At sea level less than 4 cc. of CO per kilo body weight were inhaled. At high altitude the amount in actual volume was about 5 cc. per kilo.

TABLE 4

*W. K. S.*

| DATE | R.B.C. | HEMOGLOBIN PER 100 CC. BLOOD | HEMATOCRIT (RED CELLS) | CARBON MONOXIDE METHOD | | | | DYE METHOD | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Red cell volume | Blood volume | Plasma volume | Total body hemoglobin | Plasma volume | Blood volume | Red cell volume |
| *1921* | *thousands* | *gms.* | *per cent* | *cc.* | *cc.* | *cc.* | *gms.* | *cc.* | *cc.* | *cc.* |
| Sea level | | | | | | | | | | |
| 6/3 | 5277 | | 43.2 | | | | | 3640 | 6405 | 2765 |
| 6/4 | | 15.8 | 45.3 | 2185 | 4823 | 2638 | 762 | | | |
| 6/10 | 5222 | 15.7 | 46.2 | 2217 | 4799 | 2582 | 753 | | | |
| 6/12 | | | 46.0 | | | | | 3305 | 6120 | 2815 |
| 6/14 | | | 45.2 | | | | | 3220 | 5875 | 2655 |
| 6/16 | 5181 | 15.5 | 45.5 | 2116 | 4650 | 2534 | 721 | | | |
| Average | 5227 | 15.7 | 45.2 | 2173 | 4757 | 2585 | 745 | 3388 | 6133 | 2745 |
| High altitude | | | | | | | | | | |
| 6/25 | 5308 | 16.1 | 46.3 | 2000 | 4320 | 2320 | 696 | | | |
| 7/5 | 5943 | 16.1 | 48.4 | 2565 | 5300 | 2735 | 853 | | | |
| 7/15 | 5570 | 17.5 | 46.5 | 2575 | 5538 | 2963 | 969 | | | |
| 7/18 | | | 46.5 | | | | | 3225 | 6030 | 2805 |
| 7/21 | 5937 | 19.3 | 49.8 | 2575 | 5171 | 2596 | 998 | | | |
| Sea level | | | | | | | | | | |
| 8/4 | 5920 | 17.2 | 49.0 | 2530 | 5163 | 2633 | 888 | | | |
| 8/12 | 5309 | 17.2 | 47.2 | 2430 | 5148 | 2718 | 885 | | | |
| 8/16 | 5456 | 15.5 | 45.8 | 2145 | 4683 | 2538 | 726 | | | |

Male, Weight 65 kgm.

June 18: Left San Francisco and arrived at Long Lake on June 21. For the first week there was shortness of breath on exertion and some dizziness but no cyanosis. Headache occasionally in morning and often following severe exertion. No symptoms from dye injection or from administration of carbon dioxide.

July 25 to 27: Return trip to San Francisco.

When reduced to standard conditions it was slightly under 3.5 cc. The small amount of residual CO left in the respiratory chamber and lungs was calculated and corrected for in estimating the total amount of the CO inhaled.

INDIVIDUAL CLINICAL CONDITIONS. The experimental results differ somewhat in different individuals. Shortly after arrival in camp the

two women in the party suffered from violent upper respiratory infections. They had sore throat with fever and headache. The symptoms were not simply those of mountain sickness. It seems likely that this illness may have influenced their blood volume reactions considerably for they reacted less than the four men. Evidence of other workers indicates that the blood changes in women should differ in no essential respect from those in men (6). Aside from these differences all members of the party showed quite similar changes. Fluctuations in individual experiments are inevitable and can be explained in great part by experimental error. These fluctuations are in part eliminated in table 7 where we present averages for the individual protocols of 5 of the members of the party. The sixth member (R.S.B.), one of the women, is omitted from the table of averages on account of the fact that determinations were not done at comparable intervals of time. The protocol of this case (table 5) differs from the other only in that it shows less marked reaction to altitude. In this individual the preliminary period of illess was especially severe and experiments were altogether discontinued for about 2 weeks. Furthermore the dye used for blood volume determination proved to be particularly toxic in her case; hence these determinations were discontinued entirely. The carbon monoxide gas also produced considerable headache and because of this the number of experiments was reduced to a minimum.

*Data of the experiments.* Tables 1 to 6 give the detailed experimental data for each of the members of the party. They are accompanied by a brief account of the time spent at Long Lake and of the effect of the altitude and the experimental procedures on the various individuals.

Table 1, H. R. A. At the end of the month spent at 11,000 feet the hemoglobin was 21 per cent, the R.B.C. count 16 per cent, the hematocrit 12 per cent and the R.B.C. volume (CO method) 15 per cent higher than the average sea level values. The plasma volume (dye method) was unchanged.

Table 2, A. E. B. At the end of one month on the mountains the hemoglobin was 12 per cent, the R.B.C. count 19 per cent, the hematocrit 10 per cent and the red cell volume 35 per cent higher than at sea level. The plasma volume showed almost a 6 per cent increase, but this is well within normal daily fluctuations.

Table 3, H. P. S. After six weeks at 11,000 feet elevation the hemoglobin was 5 per cent (after 17 days it reached 12 per cent and then fell again), the R.B.C. count 10 per cent, the hematocrit 8 per cent, and the red cell volume 12 per cent higher than the average at sea level. The plasma volume showed an 8 per cent drop. The final figures on this subject are not so high as some obtained earlier. He lost a little weight also and was fatigued rather than rested at the end of the six weeks. (The weight of the other members of the party remained constant.)

Table 4, W. K. S. After one month at Long Lake the hemoglobin was 23 per cent, the R.B.C. count was 13 per cent, the hematocrit 10 per cent and the red cell volume 19 per cent higher than at sea level. The plasma volume was unchanged.

Table 5, R. S. B. After one month at Long Lake the hemoglobin was 9 per cent, the hematocrit 5.5 per cent and the red cell volume 8 per cent higher than at sea level. The plasma volume was not determined. A severe upper respiratory infection began the day after arrival at Long Lake. She does not show as high a rise in blood elements as would be expected.

Table 6, E. B. C. Unlike the response in the other subjects the initial response here is a slight fall in R.B.C. count, in hematocrit and in R.C. volume. This is probably due to the acute respiratory infection which the subject acquired when she first arrived at camp. At the end of four weeks all findings were similar to those at sea level with the exception of the red cell volume which showed a 14 per cent rise. This subject's atypical reaction is interesting as it may indicate the protracted influence of the brief but severe respiratory infection.

Discussion of data. *Initial rise in red blood cell count.* Most of the protocols show a very slight rise in the red cell count within 3 or 4 days after reaching high altitude. This increase in red count is only about 200,000. In individual counts this is not beyond the limits of experimental error but the fact that it occurs in 4 of the 5 cases observed adds weight to the probability that this slight rise actually took place. This rise is not evident in the table of averages (table 7) because in the case of E. B. C. there was a rather pronounced fall due presumably to her illness. The other 4 subjects all showed this slight rise in red count. However, the fact that this rise is so small is interesting in view of the notion that on passing to high altitude there is an abrupt rise of considerable magnitude. It is largely this belief which has given support to the idea that the rise in blood count is due to loss of plasma or to a redistribution of blood cells with greater numbers in the peripheral circulation, for it seems highly unreasonable that within a few hours or even within a day or two enough new red blood cells could be formed to produce such a rise in red count.

Of the previous investigations on this point the work of Gregg, Lutz and Schneider (3) may be mentioned. They studied the physiological changes in animals enclosed in chambers in which the pressure was artificially reduced. They found that within an hour many of the animals show considerable blood concentration. Some of the animals showed a rise of 20 per cent in blood count though the average was only 3 to 5 per cent.

It is well known that human subjects subjected to such sudden lowering of pressure often experience rather severe subjective symptoms. Although proof is lacking it seems possible that any abrupt concentration of blood may be associated with these disturbances resulting from a very

TABLE 5

*R. S. B.*

| DATE | R.B.C. | HEMOGLOBIN PER 100 CC. BLOOD | HEMATOCRIT (RED CELLS) | CARBON MONOXIDE METHOD | | | | DYE METHOD | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Red cell volume | Blood volume | Plasma volume | Total body hemoglobin | Plasma volume | Blood volume | Red cell volume |
| *1921* | *thousands* | *gms.* | *per cent* | *cc.* | *cc.* | *cc.* | *gms.* | *cc.* | *cc.* | *cc.* |
| 6/4 | | | 42.6 | | | | | 2849 | 4964 | 2115 |
| 6/6 | 4596 | 16.4 | 43.2 | 1680 | 3889 | 2209 | 638 | | | |
| 6/10 | 4856 | 15.2 | 43.4 | 1675 | 3861 | 2186 | 587 | | | |
| 6/12 | | | 41.4 | | | | | 2800 | 4780 | 1980 |
| Average | 4726 | 15.8 | 42.7 | 1678 | 3875 | 2198 | 613 | 2825 | 4872 | 2046 |
| High altitude | | | | | | | | | | |
| 7/16 | 5253 | 14.8 | 44.0 | 1735 | 3943 | 2208 | 584 | | | |
| 7/24 | 5310 | 17.2 | 45.0 | 1806 | 4014 | 2208 | 690 | | | |
| Sea level | | | | | | | | | | |
| 8/4 | 5141 | 16.1 | 44.2 | 1725 | 3903 | 2178 | 628 | | | |
| 8/26 | 4686 | 14.6 | | | | | | | | |

Female, weight 61 kgm.

June 18: Left San Francisco and arrived at Long Lake June 21. There was only a mild headache at first but on the following afternoon there was rather severe headache. She awoke on the morning of the 24th with severe headache and sore-throat. Symptoms became worse during the course of the day and were accompanied by pulse rate of 120 and temperature of 101°F. Slept poorly that night. Considerable cyanosis. Pain developed in the lower part of the thorax on each side but no abnormalities made out on percussion or auscultation. No cardiac abnormalities except that pulse remained fast. On the 25th headache and sore throat continued. There were also excruciating muscle pains and in the cervical region a few enlarged lymph glands. Some earache on the 27th and 28th. From the 26th onward recovery was gradual and patient was up and about and in good spirits on the 29th. This subject suffered rather severe headaches from the carbon monoxide. The injection of brilliant vital red on June 4 caused severe symptoms. No abnormality was noted for 30 minutes. Then sharp precordial pain; pain in joints and occipital region. Stabbing pain in right side a little later. Headache, weakness and joint pains lasted several hours. There was some cardiac irregularity associated with these disturbances. On account of these symptoms the dye method was discontinued on this subject.

July 25 to 27: Return to San Francisco.

rapid fall in barometric pressure and like similar blood changes in shock are not to be regarded as true beneficial adaptation to the lowered oxygen tension. In our experiments this transition was less abrupt and sub-

jective symptoms were at no time severe. The slight rise in red count which we found is probably simply a part of the slow gradual rise which

TABLE 6

*E. B. C.*

| DATE | R.B.C. | HEMOGLOBIN PER 100 cc. BLOOD | HEMATOCRIT (RED CELLS) | CARBON MONOXIDE METHOD | | | | DYE METHOD | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Red cell volume | Blood volume | Plasma volume | Total body hemoglobin | Plasma volume | Blood volume | Red cell volume |
| *1921* | *thousands* | *gms.* | *per cent* | *cc.* | *cc.* | *cc.* | *gms.* | *cc.* | *cc.* | *cc.* |
| Sea level | | | | | | | | | | |
| 6/3 | 4901 | 14 8 | 46.4 | 1810 | 3901 | 2091 | 577 | | | |
| 6/4 | | | 42.6 | | | | | 3265 | 5690 | 2425 |
| 6/8 | 4856 | 15.1 | 43.8 | 1790 | 4087 | 2297 | 617 | | | |
| 6/9 | | | 42.9 | | | | | 3480 | 6095 | 2615 |
| 6/14 | | | 43.1 | | | | | 3030 | 5325 | 2295 |
| Average | 4878 | 15.0 | 43.8 | 1800 | 3994 | 2194 | 597 | 3258 | 5703 | 2445 |
| High altitude | | | | | | | | | | |
| 7/7 | 4684 | 15.1 | 40.4 | 1700 | 4205 | 2505 | 635 | | | |
| 7/16 | 4797 | 17.2 | 41.0 | 1840 | 4488 | 2648 | 772 | | | |
| 7/21 | | | 41.9 | | | | | 3175 | 5465 | 2290 |
| 7/23 | 4760 | 15.1 | 41.5 | 1750 | 4215 | 2465 | 637 | | | |
| 7/28 | 4948 | 15.4 | 43.7 | 2060 | 4714 | 2654 | 726 | | | |
| Sea level | | | | | | | | | | |
| 8/10 | 4792 | 14.6 | 45.6 | 1980 | 4342 | 2362 | 634 | | | |
| 8/18 | 4608 | 15.1 | 44.7 | 1675 | 3747 | 2072 | 566 | | | |
| 9/9 | 4850 | | 44.0 | 1700 | 3864 | 2164 | | | | |
| 9/23 | 4757 | | 42.0 | 1695 | 4036 | 2341 | | | | |

Female, weight 57 kgm.

June 27: Left San Francisco and arrived at Long Lake on June 29. No subjective symptoms at first except some shortness of breath with exertion. Next morning there was slight headache and nausea. Sore-throat developed in the course of the afternoon. This and the headache became worse in the next 24 hours. Confined to bed from July 1 to July 4. Headache and sore-throat continued. Temperature sometimes as high as 103°F. There were also pains in back, joints and muscles. Some diarrhea. Up and about camp on the 5th of July. Continued to feel somewhat weak for a day or two. These disturbances were somewhat similar to those experienced by R. S. B. during the first few days at Long Lake and were in all probability due to an acute upper respiratory infection. This subject was particularly free of disturbances following injections of brilliant vital red or administration of the carbon monoxide.

August 2 to 4: Return trip to San Francisco. August 20, left San Francisco for Copenhagen, Denmark and the remaining experiments were performed there in the laboratory of Prof. Kund Faber, Rigshospital.*

* E. B. C. wishes to acknowledge the kindness of Professor Faber and his laboratory staff for facilities and assistance.

goes on steadily for about two weeks. Certain of the members of the Pike's Peak expedition (2) showed a sudden rise on reaching high altitude but in these experiments also the ascent of the mountains was quite abrupt and such changes may very well be regarded as due to pathological loss of plasma and in no sense a physiological adaptation.

*Final change in R.B.C. count and in hemoglobin: color index.* On an average our experiments show within four weeks a rise in red count from 5,129,000 to 5,754,000. The increase of 625,000 is about 12 per cent of the original value. The rise in hemoglobin is from 15.4 to 17.5 grams per 100 cc., an amount equal to about 14 per cent of the original value. Thus the rise in red count and in hemoglobin are almost equal, or if anything the rise in hemoglobin is slightly greater. This is merely

TABLE 7

*Averages for H. R. A., A. E. B., E. B. C., H. P. S. and W. K. S.*

| DATE | R.B.C. | HEMOGLOBIN PER 100 CC. BLOOD | HEMATOCRIT (RED CELLS) | CARBON MONOXIDE METHOD | | | | DYE METHOD | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Red cell volume | Blood volume | Plasma volume | Total body hemoglobin | Plasma volume | Blood volume | Red cell volume |
| *1921* | *thousands* | *gms.* | *per cent* | *cc.* | *cc.* | *cc.* | *gms.* | *cc.* | *cc.* | *cc.* |
| Sea level | | | | | | | | | | |
| | 5129 | 15.4 | 44.9 | 2052 | 4484 | 2432 | 692 | 3457 | 6187 | 2730 |
| High altitude | | | | | | | | | | |
| 1st week | 5195 | 15.8 | 45.1 | 2035 | 4493 | 2458 | 710 | | | |
| 2nd week | 5638 | 17.0 | 47.5 | 2314 | 4852 | 2539 | 827 | | | |
| 3rd week | 5553 | 17.1 | 46.5 | 2316 | 4963 | 2647 | 847 | 3354 | 6071 | 2717 |
| 4th week | 5754 | 17.5 | 48.2 | 2447 | 5063 | 2616 | 867 | | | |
| Sea level | | | | | | | | | | |
| 1st week | 5374 | 16.9 | 47.9 | 2406 | 4988 | 2582 | 845 | | | |
| 2nd week | 5192 | 16.6 | 45.9 | 2249 | 4877 | 2628 | 810 | | | |
| Final | 5161 | 15.5 | 44.2 | 2006 | 4559 | 2551 | 707 | | | |

equivalent to the statement that the color index is practically unchanged. Previous workers have differed on this point. Some obtained a slight rise; others found a slight fall. In our series the individual cases vary but in no instance is there any very clear evidence of a marked change in either direction. The case of E. B. C. differs from the others in regard to the rise in red cells and hemoglobin but it will be recalled that in her case the original high-altitude value is lower than the previous sea level values, due presumably to the illness already noted. When this original fall is taken into account it is seen that regeneration goes on, never, however, to a value much above the original sea level figure.

The atypical reaction on the part of E. B. C. affects the table of averages considerably. If the four male members of the party alone are considered the average per cent rise in red count is about 15 per cent. The average per cent rise in hemoglobin for the four men is also the same —15 per cent. Examination of the individual protocols shows that in some cases the percentage rise in hemoglobin is greater than the rise in red blood cells; in other cases the reverse is true. Later the possible explanation for this individual variation will be discussed.

*Change in the red cell volume.* The rise in red cell volume was not demonstrable until two or three weeks had passed. The changes indicated by the carbon monoxide method are quite similar in most of the experiments. The averages indicate an increase in red cell volume from 2052 to 2447 cc. This is about 19 per cent of the original value. Obviously the rise would be even greater if the hematocrit had shown a rise in proportion to the rise in red count or hemoglobin. The time which the majority of the party spent at Long Lake was not sufficient to demonstrate that they had reached a maximum cell volume. In four of the subjects the last red cell volume reading was the highest. In W. K. S. however a plateau was reached early. In the case of H. P. S. six weeks instead of four were spent at Long Lake, but the cell volume did not increase after the fourth week. It seems probable, therefore, at least in the case of the men, that the last reading for red cell volume represents the maximum volume which could be reached at that altitude.

*Plasma volume findings.* The plasma volume showed a slight increase in the case of A. E. B. and a slight decrease in the case of H. P. S. but no fluctuation in the other members of the expedition.

While the individual protocols agree in consistently showing a rise in all four elements, hemoglobin, R.B.C. count, hematocrit and R.B.C. volume there is at first what seems to be a disturbing difference in the relative increase in the four elements. Thus H. R. A. has the highest per cent of rise in his hemoglobin, while the red cell count and the red cell volume are practically the same and hematocrit is 3 per cent lower. A. E. B. has a very great increase in red cell volume and only a 10 per cent increase in hematocrit. H. P. S. shows only a moderate increase in all elements. The blood volume is the greatest, while in W. K. S. the hemoglobin and red cell volume increase considerably more than the other two elements. In the case of R. S. B. the red cell count has the greatest per cent rise. Some of the fluctuations are undoubtedly due to experimental error. It must be remembered that the blood volume methods may have as much as a 5 per cent error. In table 7 where the data of the experiments are averaged these differences are very much reduced and the error undoubtedly lessened. However, all the fluctuations cannot be explained on such a basis.

Before discussing the possible explanations for the individual variations it would be well to have clearly in mind what effect on red cell count and hematocrit a given rise in total red cell volume would have. Given a blood volume of 2000 cc. and a hematocrit of 50 per cent, if the cell volume increases to 1200 cc. (20 per cent) while the plasma volume remains constant the hematocrit will increase to 54.5 per cent. This is a rise of only 9 per cent above the original hematocrit value. If the new cells are of the same size as the old the red cell count will increase by the same per cent as the hematocrit. The same is true of the per cent hemoglobin provided each new cell contains the same amount of hemoglobin as the old ones.

But if the new cells added are smaller the cell count would rise out of proportion to the hematocrit, and in the same way the hemoglobin might vary from the expected amount because the new cells might not contain the same amount of hemoglobin as the old ones.

Another factor which must be kept in mind is that the new blood more concentrated in cells may not be mixed in the same proportions throughout the circulatory system. In this case the change in hematocrit would not be comparable to that in total cell volume. If these variable factors are kept in mind while considering the data presented by our experiments it is readily understood that the per cent rise in hematocrit is much lower than the per cent rise in red cell volume. It also is reasonable to find that there is considerable individual variation in the change in the various elements of the blood. The general response is evidently an increased production of red blood cells—but each subject of the party did not show the same degree of activity in this respect nor were the type of cells as regards size and hemoglobin content always the same. The highest rise in red cell volume occurred in A. E. B.—a rise of 35 per cent with only a 10 per cent increase in hematocrit, where we might have expected about a 16 per cent rise. It is to be noted however that in this individual there is also a slight rise in plasma volume and this would considerably reduce the expected per cent of hematocrit.

*Change in red cell count and hemoglobin with relation to hematocrit readings.* The hematocrit at sea level was 44.9 per cent and after four weeks at high altitude it rose to only 48.2 per cent. This is equivalent to 3.3 parts in 44.9 or a little over 7 per cent. It is evident that the rise in hematocrit is less than the rise in red count or hemoglobin which amounted to about 12 per cent. Had the hematocrit risen in proportion to the red cells or hemoglobin the hematocrit at the end of four weeks would have been about 50.7 per cent cells instead of the observed 48.2 per cent. This difference is beyond the limits of experimental error. As has been stated the centrifuge used in these experiments was carefully standardized. Variations in individual readings taken on the same blood rarely exceed

2 parts in 50 of packed cells. In the present series the micro-hematocrits were always run in duplicate, thus somewhat reducing this error. It therefore is necessary to conclude that the red cells become smaller in size. From our experiments there is no way of knowing whether all of the red cells or only those which are formed after arrival at high altitude participate in the process. In no case were any very small cells noted while making the blood counts, nor in fact were any irregularities in the size of cells observed. It seems quite likely nevertheless that the newly-formed cells are slightly undersized and that as the old cells degenerate and are destroyed they are gradually replaced by others of the smaller variety. It is noteworthy that Sundstroem and Bloor (8) found shrinkage in size of red blood cells in animals subjected to reduced atmospheric pressures in closed chambers. Their experiments were conducted over a relatively brief period of time and they look upon such shrinkage as the result of a disturbance in the acid-base equilibrium. In our experiments the change in pressure was so much more gradual that the disturbance in acid-base equilibrium must have been slight at any one time. Furthermore our tables do not show shrinkage in red cells immediately on reaching high altitude as would be the case if the shrinkage arose from a sudden disturbance in the acid-base equilibrium. In our first determinations at high altitude the cells are of almost exactly the same size as had been found at sea level. Later the cells became gradually smaller and smaller so that apparently the shrinkage results from the gradual replacement of old cells by smaller new ones.

This change in size of red cells has been considered by previous investigators. In most instances the size of the individual cells was determined by direct measurement under the microscope. This proved a rather unreliable method and conflicting results were obtained by the various workers. In the paper of Douglas, Haldane, Henderson and Schneider (2) a few hematocrit determinations are given. These few figures agree with our figures in showing that after a period of residence at high altitude the new cells are somewhat smaller than are the cells of the same subject at sea level. They had so few determinations that they were not inclined to attach much significance to the change but even these few figures are substantially in agreement with the figures presented in the present paper.

It may seem somewhat remarkable that despite the general shrinkage in size of individual red cells, nevertheless each cell now contains as much hemoglobin as at sea level. From the figures given in the table of averages it is seen that at sea level 100 cc. of blood contained 15.4 grams of hemoglobin. Since the hemoglobin is confined to the red cells it is obvious, too, that 44.9 cc. red cells contained 15.4 grams of hemoglobin, or that 100 cc. of cells would contain 34.3 grams of hemoglobin. Calcula-

tions made in the same way show that after four weeks at high altitude 100 cc. of cells contained 36.3 grams of hemoglobin, a rise of 6 per cent, which is beyond the limits of experimental error. By similar calculation 100 cc. of cells at sea level contained 1140 billion individual cells while 100 cc. of cells at Long Lake contained 1190 billion individual cells. This is an increase of about 4.5 per cent in the total number of cells per 100 cc. Therefore each individual cell, although smaller, contains as much or more hemoglobin than at sea level. The hyper-pigmentation of the red cell stroma may seem less surprising if we consider it as an adaptation whereby more oxygen can be carried to the tissues in the minimum bulk of the red cell stroma. Likewise we may look upon the smaller size of the red cells as an adaptation giving relatively larger surface area.

*Total number of red blood cells at high altitude.* Another point worth special attention is the result of the fact that the red cells are smaller at high altitude than at sea level. From this it follows that the increase in number of red cells is disproportionately great when compared to the increase in total red cell volume. Thus from the table it is easily shown that the 2052 cc. of cells present at sea level are formed by the presence of 23.4 trillion red cells. At high altitude similar computation indicates that the body contained 29.1 trillion cells. The increase of 5.7 trillion cells is an increase of about 24 per cent over the original number.

Thus to summarize, our evidence indicates that after four weeks' residence at 11,000 feet the red count and hemoglobin rise about 12 per cent above their original sea level values. The evidence indicates that the red cells are slightly smaller but their stroma sufficiently richer in hemoglobin to compensate for the diminished size of the cell. The total red cell volume rises about 19 per cent above its original value and the evidence seems to indicate no change in the plasma volume.

The changes associated with return to sea level call for little discussion. In every case the final determinations correspond quite closely with the original determinations at sea level. This change occurred gradually and in most instances was not quite complete within 2 weeks after return to sea level. This gradual return to the original value is particularly well shown in the figures for red cell volume. Within two weeks practically half of the gain had been lost.

## SUMMARY

The effect on hemoglobin, red blood cells, hematocrit and blood volume of four weeks residence at high altitude was studied in 6 human subjects.

The red count and hemoglobin rise almost proportionately throughout, reaching in 3 weeks a maximum of 10 to 15 per cent above the sea level value.

The percentage of red blood cells (hematocrit) rises somewhat less indicating that the individual cells are slightly smaller though capable of holding the same amount of hemoglobin.

There is no evidence of abrupt changes in red blood cells or blood volume within the first day or two spent at high altitude.

Blood volume determinations by the dye and by the carbon monoxide methods indicate that the rise in red cells and hemoglobin is due to the production of more cells, not to a redistribution.

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# THE GROWTH AND AGE INVOLUTION OF THE THYMUS IN MALE AND FEMALE PIGEONS

OSCAR RIDDLE AND PAUL FREY

*From the Carnegie Station for Experimental Evolution, Cold Spring Harbor, N.Y.*

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In a recent paper (1) one of us has presented evidence that the thymus is essential to the production of the envelopes which enclose the bird's egg. It was there also pointed out that the thymus probably has a similar function in those animals—all vertebrates except mammals—which retain the usual or primitive vertebrate type of reproduction. The results of that study serve to increase the desirability of definite information concerning the growth and the adult history of the thymus in vertebrates other than mammals. One would particularly wish to know how the thymi of birds and lower vertebrates compare in size with the mammalian thymus; whether its growth and its age involution occur at a similar time and to a similar degree in mammalian and non-mammalian forms; and finally, whether this organ shows any sexual difference in those animals in which the thymus apparently exercises an endocrine function in connection with the formation of egg-albumen and egg shell. The present paper presents the data on these subjects as obtained by us chiefly in three kinds of pigeons. It is thought that measurements of the growth and extraordinary size fluctuations of the thymus may ultimately contribute much toward a better understanding of the physiology of this organ.

*Earlier observations.* Some of the earliest observations on the thymus of fishes record the absence of this organ in the largest specimens of several species of teleosts. At that time, however, no attention was given to the ready involution of the thymus in disease, fasting, and other adverse conditions; it therefore now seems uncertain whether such absence in very old fishes is normal and general. Later observations have usually indicated that the beginning of thymic involution occurs later in fishes than in mammals. Prymak (2) says that "the involution of the thymus in these vertebrates (teleosts) occurs in all cases but relatively much later than in mammals."

Hammar (3) found it difficult to declare the precise period of age involution in teleosts, but thinks it occurs at a relatively late stage and possibly coincident with sexual maturity. He observed that a fasting period of 23 to 31 days reduced the teleost thymus to one-sixth its normal size.

In a study of two Elasmobranch species Hammar (4) also found that involution begins at or near sexual maturity. The extent to which size reduction continues in old age was not learned, but the relative amount of this reduction which immediately followed the beginning of involution seems quite similar to that shown by our curves in pigeons. Indeed Hammar states that in the time of its appearance and in the early course of its progress thymic involution in these lowest forms notably resembles that of mammals.

If studies have been made on the growth or on the age involution of the thymus in Amphibia and Reptilia they are wholly unknown to us. The observations of Aimé (5) on the annual size fluctuations of the turtle's thymus would seem, however, to imply a fair degree of thymic persistence in this reptile. It was observed that the thymus becomes greatly reduced during the winter; and that annually, from spring to autumn, a remarkable new growth occurs which the author states may be regarded as a repetition of the embryonic development of the organ.

According to Paton (6), Soli has stated (place not cited) that thymic involution does not occur in birds. Paton further says that his own casual observations on ducks lead him to doubt this conclusion. We have been unable to find or to verify the statement credited to Soli, or to learn definitely of the material on which such a statement may have been based. So far as known to us, the only birds studied by Soli were fowls, and the thymus was examined in only a few cases. Jolly (7) tabulated the thymus weights of 21 fowls, aged 2 to 12 months, but noted that only insufficient data, including his own, were then available for any kind of bird. Jolly was inclined to think, however, that the thymus here involutes quite slowly and is much influenced by nutritive and "seasonal" changes.

McCarrison (8), (9) has recently reported some data of importance on the size of the thymus in common pigeons, and concerning the causes of its accidental or temporary involution. His data, though inadequate, exceed in amount all of the previous thymus data for all birds. The male thymus was found to be twice as large as that of the female. Disease, fasting and avitaminosis were found to cause a rapid disappearance of this gland in these birds. The material studied consisted of 30 healthy males and 27 healthy females together with a number of diseased and treated (avitaminosis) individuals. The age of most of these birds was known (61 days—after hatching?—to more than 2 years) but individuals were tabulated only in relation to body weight, not age, and no attempt was made to construct curves of growth or of involution. The evidence was considered sufficient, however, to permit the conclusion that only a delayed or a restricted involution of the thymus occurs in the pigeon. Much that is necessary to a comprehension of the conditions found in all kinds of pigeons, together with the chief results of McCarrison's study which relate to the present communication, is contained in the following quotations.

In the healthy birds the thymus varies considerably in size, and in three cases out of thirty-five controls it existed only in traces which were not weighable. Its variations in the male ranged from mere traces to 1,702 mgm.; in the female from traces only (2 cases) to 776 mgm. The gland is slightly more than twice as large in healthy males as in healthy females. The average weight in the former is 678 mgm., in the latter 318 mgm.

In diseased birds the thymus atrophies out of all proportion to the body weight. In males its average weight is reduced to 14 mgm. The merest traces only (and these unweighable) could be found in thirteen of the diseased birds; in the remaining 7 birds, in which a weighable thymus was dissected out, the weight of the organ ranged from 15 to 85 mgm. In diseased females, on the other hand, the gland had completely disappeared in the present series, or existed only as a thin thread of tissue closely applied to the great vessels of the neck. The thymus was examined histologically in a fair sample of these birds without revealing any notable evidence of the replacement of thymic tissue by fat, connective tissue or pathological deposits. . . . .

In healthy males it is rare to find the thymus so small that it exists in traces only. Indeed, my experience leads me to believe that such rare instances, in apparently healthy birds, may be examples of diseased states which my methods of observation have failed to reveal. . . . .

In so far as pigeons are concerned, the involution of the thymus is dependent on factors other than maturation of the sexual function: these factors are mainly nutritional.

It may possess some relationship to the sex-glands since it is twice as large in males as in females, and is influenced adversely by the same adverse circumstances.

It was earlier noted by one of us (1), "that although the thymus by no means disappears in the adult pigeon, it does become markedly and progressively reduced in old age." Very few data on this point were there cited. The data contained in the present paper supplied the basis for that statement.

*Material.* Males and females of three kinds of pigeons and doves have been examined at intervals covering the periods of growth and involution. For healthy, for *Ascaridia*-bearing, and for dead individuals of these three kinds the weights of 903 pairs of thymi have been tabulated. Some additional data are given in the text. Thymus weights have been obtained in considerable numbers for several other kinds of pigeons—particularly on various kinds of hybrids—but very few data are available for healthy young of any of these groups. The common pigeons used by us are essentially the same birds as are widely used as laboratory animals, and are probably comparable in kind to those used by McCarrison. The ring doves used here are the two common species of cage ring doves (*Streptopelia risoria* and *St. alba*) and their hybrids.

A third group, here called "ring dove hybrids" are the doves last mentioned above into which a trace (always less than one-eighth) of the blood of a different genus (*Turtur orientalis*) has been added. This is by far the least homogeneous material tabulated; it nevertheless yielded results

quite similar to those obtained from the other two groups. Neither of these three groups was composed of genetically similar individuals but this seems of doubtful importance. The various strains or races included in any one group, except in the case noted above, were of fairly uniform body weight. Further excepting the probability that the younger the age-group the greater the amount of inbreeding involved, the material used seems wholly comparable. The amount of data tabulated here for pigeons is apparently four or five times the amount previously available for fowls, pigeons and all other birds combined. Birds of all ages are represented, and the attempt was made to obtain a series of thymus weights which as nearly as possible represent normal and healthy states of the animal.

The exact age of every bird was known, and age is calculated from the date the egg is laid, not from the date of hatching. Thus ring doves are 0.5 month (14 to 15 days) old at hatching; at the same period common pigeons are 0.6 month (17 to 18 days) old. Our practice is to remove all young from their parents when about 1.3 or 1.4 months old; following this cessation of parental feeding it probably often happens that a short period of underfeeding results. A very few young, with body and thymus weight thus temporarily reduced, are doubtless included in age groups from 1.3 to 1.7 months old. From 1.4 months to about 5 or 6 months all birds, though kept in confinement, were given the freedom of much larger cages than those later used throughout the period of maturity. If, as seems possible, confinement in itself tends to induce thymic involution, this process has been accentuated in birds older than 5.0 months by our procedures. The tabulated data show conclusively, however, that in all three kinds of pigeons the beginning of age involution occurred prior to 5.0 months, and therefore before they were placed (in pairs) in smaller pens ($2 \times 5 \times 8$ feet).

Nearly all of the present data for birds aged more than 6 months have been obtained in connection with other endocrine and sex studies, and therefore from birds killed at all seasons and months of the year. A part of the data for younger birds was similarly obtained but most of these young were killed during September and early October. The thymus has been observed and recorded in terms of comparative size ("very large," "large," "small," etc.) in more than 1,000 pigeons not included in the present data. The present data of course include only actual weighings of thymi carefully freed of adjacent fat. It is not yet known whether in our material the thymus is affected by season. All birds used here had been protected against extreme cold in heated buildings.

It is practically certain that not all diseased birds have been recognized as such by us. This would tend to make our figures for the healthy thymus too low. But birds much under weight, those showing intestinal stasis or fermentation, those obviously tuberculous, those bearing *Ascaridia*,

and others much affected with a *Trichuris*-like parasite, have been recognized and excluded. Those whose intestines contained *Ascaridia* have been separately tabulated. Birds aged less than 2.0 months, and unhealthy from any source other than recognizable tuberculosis, have been classed with the *Ascaridia*-infested young.

In general birds aged 6.0 to 6.9 months, 7.0 to 8.9 months, 9.0 to 16.9 months, and 20.0 to 29.9 months, have been united to form groups of approximately 6, 8, 12 and 24 months. Younger groups were obtained in a similar way; for example, birds were killed at 2.1 to 2.6 months to provide a group whose age usually averaged about 2.4 months.

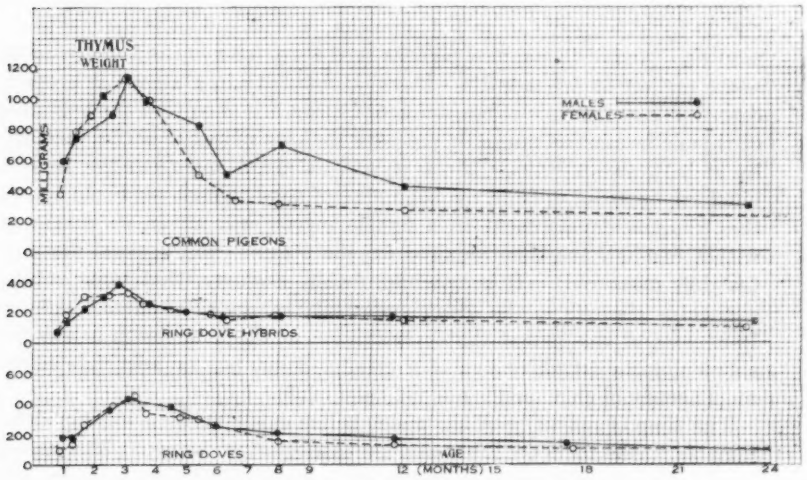


Fig. 1

*The data for growth and for age involution.* The main results concerning the growth and involution of the pigeon thymus during the first 24 months of life can be readily seen on the curves of the chart. The curves are formed from data for healthy birds only, and have been constructed for the three kinds of pigeons on which we have the most data (tables 1 to 3). It will be observed that the maximum size of the thymus is attained at about 3.0 months (2.5 months after hatching) or very soon thereafter. Reference to tables 1 to 3 will show that the adult body weight is also attained at practically this same period. The figures given for "milligrams thymus per 100 grams body weight" in these tables also show that age-groups older than 3.0 months have progressively less thymus per unit of body weight. Preceding the 3.0 month stage the figures for this ratio are variable. At the 3.0 month stage the proportion, as well as the absolute amount, of thymic tissue is usually at its highest.

In order to fix the maximum size of the thymus with reference to the attainment of sexual maturity some further data must now be supplied. The youngest age at which any pigeon of our collection ever has laid her first egg is 4.0 months. This record is the same for a common pigeon and for a ring dove. The usual age of sexual maturity in our birds, however, lies between 5 and 8 months in both these groups of birds. The earliest age that may be considered the usual or expected time of attainment of the capacity to produce eggs is 5.5 months; probably this average time is as much as 6.0 months. On this point we may give more specific data for some of the particular birds whose thymus weights are presented. Of the seven ring doves aged 4.0 to 5.4 months none had yet laid an egg. Of the 20 hybrids aged 4.0 to 5.8 months, 9 had laid—the earliest at 5.0 months. And of 13 common pigeons aged 4.0 to 5.8 months 5 had ovulated —the earliest at 4.5 months. If sexual maturity in a pigeon is properly describable as the time of beginning ovulation it is clear that the beginning of thymic involution normally precedes the attainment of sexual maturity by at least two months. If one should calculate age from the time of hatching—instead of from the beginning of development—it could be said that thymus development in a female pigeon will attain its maximum within one-half the time required for the attainment of sexual maturity (as thus measured). It is not improbable, however, that some notable change or changes in the ovary occurs at the age of about 3.0 months. It is close to this period that examination of the ovary with a lens first shows minute definitive ova of a creamy color, but no adequate microscopic study of this matter has yet been made.

The time of maturity of the males is less easily and less exactly determinable. The facts on this point are inadequately known. The growth curve of the pigeon testis is known from unpublished data to differ considerably from the growth curve of the mammalian testis. It is known to continue to increase markedly in size long after the beginning of sperm formation. In the healthy males of the 3.1 month group of table 1—the group showing maximum thymus size—the average weight of the two testes of each bird was 0.283 gram; this is approximately one-eighth the amount of testis usually found in a normal male aged 1 to 2 years. Our experience suggests that some of these testes would not have delayed for long the production of mature sperm; but it is possible that sperm production would have appeared only at as late an age as does sexual maturity in the females. Among these common pigeons killed at less than 6.0 months the youngest male known to be producing sperm was one of two (with *Ascaridia*) aged 4.5 months.

From the above considerations it is evident that the 24 months which mark the oldest age limit shown in the curves is approximately four times the period of sexual maturity. It thus corresponds to perhaps 50 or 60

TABLE 1

*Thymus size in relation to age and sex in common pigeons*

| CONDITION | SEX | AGE | NUMBER OF BIRDS | WEIGHT OF: | | MILLIGRAMS THYMUS PER 100 GRAMS BODY WEIGHT |
|---|---|---|---|---|---|---|
| | | | | Body | Thymus | |
| | | *months* | | *grams* | *mgm.* | |
| Healthy | Males | 1.0 | 3 | 172 | 599 | 348 |
| | | 1.4 | 4 | 303 | 740 | 244 |
| | | 2.6 | 3 | 286 | 891 | 312 |
| | | 3.1 | 6 | 332 | 1,148 | 346 |
| | | 3.7 | 4 | 327 | 985 | 301 |
| | | 5.4 | 2 | 395 | 825 | 209 |
| | | 6.3 | 7 | 332 | 512 | 154 |
| | | 8.1 | 8 | 342 | 692 | 203 |
| | | 12.1 | 39 | 337 | 424 | 126 |
| | | 23.3 | 10 | 375 | 296 | 79 |
| | Females | 0.9 | 3 | 151 | 388 | 257 |
| | | 1.4 | 7 | 273 | 785 | 288 |
| | | 1.9 | 3 | 298 | 887 | 297 |
| | | 2.3 | 3 | 267 | 1,022 | 383 |
| | | 3.0 | 1 | 307 | 1,126 | 367 |
| | | 3.8 | 3 | 349 | 993 | 284 |
| | | 5.4 | 6 | 321 | 502 | 156 |
| | | 6.6 | 8 | 309 | 338 | 109 |
| | | 8.0 | 9 | 331 | 317 | 96 |
| | | 12.1 | 31 | 324 | 268 | 83 |
| | | 24.5 | 3 | 364 | 227 | 62 |
| Ascaridia | Males | 1.0 | 2 | 149 | 349 | 234 |
| | | 1.2 | 3 | 221 | 284 | 128 |
| | | 2.1 | 5 | 279 | 347 | 125 |
| | | 3.2 | 10 | 310 | 638 | 206 |
| | | 4.5 | 2 | 357 | 532 | 149 |
| | | 5.4 | 2 | 285 | 228 | 80 |
| | | 6.0 | 2 | 332 | 447 | 135 |
| | | 8.3 | 2 | 332 | 453 | 137 |
| | | 12.8 | 24 | 360 | 413 | 115 |
| | | 22.9 | 8 | 359 | 373 | 104 |
| | Females | 1.8 | 3 | 239 | 253 | 106 |
| | | 2.6 | 4 | 295 | 476 | 161 |
| | | 3.6 | 6 | 294 | 448 | 152 |
| | | 5.2 | 4 | 266 | 311 | 117 |
| | | 5.9 | 2 | 328 | 273 | 83 |
| | | 6.6 | 6 | 330 | 213 | 65 |
| | | 8.3 | 9 | 333 | 461 | 139 |
| | | 12.7 | 30 | 325 | 235 | 72 |
| | | 23.2 | 9 | 314 | 153 | 49 |
| Dead | Males and females | 1.1 | 14 | 109 | 95 | 96 |
| | | 1.7 | 13 | 179 | 10 | 6 |
| | | 2.2 | 10 | 206 | 17 | 8 |
| | | 2.7 | 3 | 180 | 37 | 21 |
| | | 3.2 | 4 | 239 | 41 | 17 |
| | | 7.9 | 6 | 261 | 194 | 40 |
| | | 13.3 | 21 | 257 | 151 | 54 |
| | | 26.6 | 4 | 236 | 25 | 11 |

In order to fix the maximum size of the thymus with reference to the attainment of sexual maturity some further data must now be supplied. The youngest age at which any pigeon of our collection ever has laid her first egg is 4.0 months. This record is the same for a common pigeon and for a ring dove. The usual age of sexual maturity in our birds, however, lies between 5 and 8 months in both these groups of birds. The earliest age that may be considered the usual or expected time of attainment of the capacity to produce eggs is 5.5 months; probably this average time is as much as 6.0 months. On this point we may give more specific data for some of the particular birds whose thymus weights are presented. Of the seven ring doves aged 4.0 to 5.4 months none had yet laid an egg. Of the 20 hybrids aged 4.0 to 5.8 months, 9 had laid—the earliest at 5.0 months. And of 13 common pigeons aged 4.0 to 5.8 months 5 had ovulated —the earliest at 4.5 months. If sexual maturity in a pigeon is properly describable as the time of beginning ovulation it is clear that the beginning of thymic involution normally precedes the attainment of sexual maturity by at least two months. If one should calculate age from the time of hatching—instead of from the beginning of development—it could be said that thymus development in a female pigeon will attain its maximum within one-half the time required for the attainment of sexual maturity (as thus measured). It is not improbable, however, that some notable change or changes in the ovary occurs at the age of about 3.0 months. It is close to this period that examination of the ovary with a lens first shows minute definitive ova of a creamy color, but no adequate microscopic study of this matter has yet been made.

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TABLE 1

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| CONDITION | SEX | AGE | NUMBER OF BIRDS | WEIGHT OF: | | MILLIGRAMS THYMUS PER 100 GRAMS BODY WEIGHT |
|---|---|---|---|---|---|---|
| | | | | Body | Thymus | |
| | | *months* | | *grams* | *mgm.* | |
| Healthy | Males | 1.0 | 3 | 172 | 599 | 348 |
| | | 1.4 | 4 | 303 | 740 | 244 |
| | | 2.6 | 3 | 286 | 891 | 312 |
| | | 3.1 | 6 | 332 | 1,148 | 346 |
| | | 3.7 | 4 | 327 | 985 | 301 |
| | | 5.4 | 2 | 395 | 825 | 209 |
| | | 6.3 | 7 | 332 | 512 | 154 |
| | | 8.1 | 8 | 342 | 692 | 203 |
| | | 12.1 | 39 | 337 | 424 | 126 |
| | | 23.3 | 10 | 375 | 296 | 79 |
| | Females | 0.9 | 3 | 151 | 388 | 257 |
| | | 1.4 | 7 | 273 | 785 | 288 |
| | | 1.9 | 3 | 298 | 887 | 297 |
| | | 2.3 | 3 | 267 | 1,022 | 383 |
| | | 3.0 | 1 | 307 | 1,126 | 367 |
| | | 3.8 | 3 | 349 | 993 | 284 |
| | | 5.4 | 6 | 321 | 502 | 156 |
| | | 6.6 | 8 | 309 | 338 | 109 |
| | | 8.0 | 9 | 331 | 317 | 96 |
| | | 12.1 | 31 | 324 | 268 | 83 |
| | | 24.5 | 3 | 364 | 227 | 62 |
| Ascaridia | Males | 1.0 | 2 | 149 | 349 | 234 |
| | | 1.2 | 3 | 221 | 284 | 128 |
| | | 2.1 | 5 | 279 | 347 | 125 |
| | | 3.2 | 10 | 310 | 638 | 206 |
| | | 4.5 | 2 | 357 | 532 | 149 |
| | | 5.4 | 2 | 285 | 228 | 80 |
| | | 6.0 | 2 | 332 | 447 | 135 |
| | | 8.3 | 2 | 332 | 453 | 137 |
| | | 12.8 | 24 | 360 | 413 | 115 |
| | | 22.9 | 8 | 359 | 373 | 104 |
| | Females | 1.8 | 3 | 239 | 253 | 106 |
| | | 2.6 | 4 | 295 | 476 | 161 |
| | | 3.6 | 6 | 294 | 448 | 152 |
| | | 5.2 | 4 | 266 | 311 | 117 |
| | | 5.9 | 2 | 328 | 273 | 83 |
| | | 6.6 | 6 | 330 | 213 | 65 |
| | | 8.3 | 9 | 333 | 461 | 139 |
| | | 12.7 | 30 | 325 | 235 | 72 |
| | | 23.2 | 9 | 314 | 153 | 49 |
| Dead | Males and females | 1.1 | 14 | 109 | 95 | 96 |
| | | 1.7 | 13 | 179 | 10 | 6 |
| | | 2.2 | 10 | 206 | 17 | 8 |
| | | 2.7 | 3 | 180 | 37 | 21 |
| | | 3.2 | 4 | 239 | 41 | 17 |
| | | 7.9 | 6 | 261 | 194 | 40 |
| | | 13.3 | 21 | 257 | 151 | 54 |
| | | 26.6 | 4 | 236 | 25 | 11 |

TABLE 2

*Thymus size in relation to age and sex in ring doves*

| CONDITION | SEX | AGE | NUMBER OF BIRDS | WEIGHT OF: | | MILLIGRAMS THYMUS PER 100 GRAMS BODY WEIGHT |
|---|---|---|---|---|---|---|
| | | | | Body | Thymus | |
| | | *months* | | *grams* | *mgm.* | |
| Healthy | Males | 1.0 | 2 | 87 | 180 | 207 |
| | | 1.3 | 2 | 125 | 182 | 145 |
| | | 2.5 | 3 | 153 | 360 | 235 |
| | | 3.1 | 1 | 163 | 436 | 267 |
| | | 4.5 | 2 | 146 | 376 | 275 |
| | | 6.0 | 5 | 150 | 251 | 167 |
| | | 8.0 | 6 | 148 | 204 | 138 |
| | | 11.8 | 24 | 158 | 169 | 107 |
| | | 17.4 | 6 | 175 | 139 | 79 |
| | | 24.3 | 10 | 178 | 90 | 51 |
| | Females | 0.9 | 3 | 77 | 90 | 116 |
| | | 1.3 | 3 | 108 | 130 | 121 |
| | | 1.7 | 2 | 130 | 271 | 209 |
| | | 2.6 | 2 | 137 | 397 | 289 |
| | | 3.3 | 2 | 147 | 447 | 304 |
| | | 3.7 | 3 | 149 | 340 | 228 |
| | | 4.8 | 2 | 152 | 318 | 209 |
| | | 5.4 | 2 | 153 | 303 | 198 |
| | | 5.9 | 5 | 152 | 256 | 169 |
| | | 8.0 | 16 | 153 | 157 | 102 |
| | | 11.8 | 12 | 158 | 124 | 78 |
| | | 17.6 | 4 | 172 | 113 | 66 |
| | | 38.6 | 2 | 166 | 88 | 53 |
| Ascaridia | Males | 1.4 | 2 | 111 | 157 | 142 |
| | | 2.4 | 2 | 145 | 313 | 215 |
| | | 3.4 | 4 | 153 | 177 | 116 |
| | | 8.0 | 4 | 164 | 247 | 150 |
| | | 12.8 | 33 | 159 | 139 | 87 |
| | | 16.9 | 5 | 172 | 87 | 51 |
| | | 21.6 | 1 | 181 | 148 | 82 |
| | Females | 1.3 | 1 | 87 | 105 | 121 |
| | | 1.7 | 2 | 130 | 237 | 182 |
| | | 2.5 | 1 | 110 | 332 | 302 |
| | | 3.6 | 3 | 138 | 172 | 125 |
| | | 4.4 | 3 | 141 | 177 | 125 |
| | | 6.3 | 3 | 141 | 166 | 118 |
| | | 7.8 | 3 | 165 | 117 | 71 |
| | | 12.6 | 31 | 154 | 137 | 89 |
| | | 18.2 | 3 | 167 | 89 | 53 |
| Dead | Male and female | 6.5 | 3 | 124 | 78 | 63 |
| | | 7.3 | 2 | 113 | 30 | 27 |
| | | 11.0 | 4 | 118 | 60 | 51 |
| | | 26.5 | 4 | 141 | 10 | 7 |

TABLE 3

*Thymus size in relation to age and sex in ring dove hybrids (Streptopelia with traces of the genus Turtur)*

| CONDITION | SEX | AGE | NUMBER OF BIRDS | WEIGHT OF: | | MILLIGRAMS THYMUS PER 100 GRAMS BODY WEIGHT |
|---|---|---|---|---|---|---|
| | | | | Body | Thymus | |
| | | *months* | | *grams* | *mgm.* | |
| Healthy | Males | 0.8 | 5 | 59 | 76 | 129 |
| | | 1.1 | 4 | 104 | 132 | 127 |
| | | 1.7 | 5 | 134 | 225 | 168 |
| | | 2.3 | 5 | 150 | 303 | 202 |
| | | 2.8 | 3 | 144 | 376 | 261 |
| | | 3.8 | 2 | 154 | 261 | 169 |
| | | 5.0 | 1 | 152 | 203 | 133 |
| | | 6.2 | 5 | 151 | 171 | 113 |
| | | 8.1 | 27 | 159 | 171 | 107 |
| | | 11.7 | 19 | 159 | 170 | 107 |
| | | 23.5 | 4 | 164 | 144 | 84 |
| | Females | 0.8 | 2 | 58 | 77 | 133 |
| | | 1.1 | 2 | 117 | 192 | 164 |
| | | 1.7 | 3 | 128 | 307 | 240 |
| | | 2.5 | 1 | 150 | 309 | 206 |
| | | 3.1 | 3 | 156 | 322 | 206 |
| | | 3.6 | 3 | 146 | 259 | 177 |
| | | 4.5 | 7 | 150 | 222 | 147 |
| | | 5.8 | 4 | 144 | 198 | 138 |
| | | 6.3 | 11 | 154 | 152 | 98 |
| | | 7.9 | 23 | 154 | 179 | 116 |
| | | 12 1 | 18 | 160 | 152 | 95 |
| | | 23.2 | 2 | 148 | 109 | 74 |
| Ascaridia | Males | 1.2 | 3 | 109 | 66 | 60 |
| | | 1.7 | 2 | 122 | 46 | 38 |
| | | 2.5 | 2 | 153 | 175 | 114 |
| | | 3.1 | 2 | 163 | 134 | 82 |
| | | 3.9 | 1 | 117 | 201 | 172 |
| | | 5.8 | 1 | 160 | 106 | 66 |
| | | 6.5 | 2 | 153 | 221 | 144 |
| | | 8.0 | 4 | 150 | 200 | 134 |
| | | 12.0 | 13 | 151 | 176 | 117 |
| | | 21.5 | 2 | 167 | 117 | 70 |
| | Females | 1.1 | 1 | 92 | 91 | 99 |
| | | 1.7 | 4 | 121 | 232 | 191 |
| | | 2.6 | 7 | 137 | 172 | 126 |
| | | 3.0 | 5 | 150 | 222 | 148 |
| | | 3.9 | 4 | 139 | 165 | 119 |
| | | 4.7 | 4 | 151 | 136 | 90 |
| | | 5.8 | 4 | 143 | 173 | 121 |

TABLE 3—*Concluded*

| CONDITION | SEX | AGE | NUMBER OF BIRDS | WEIGHT OF: | | MILLIGRAMS THYMUS PER 100 GRAMS BODY WEIGHT |
|---|---|---|---|---|---|---|
| | | | | Body | Thymus | |
| | | *months* | | *grams* | *mgm.* | |
| Ascaridia | Females | 6.1 | 2 | 141 | 171 | 121 |
| | | 7.7 | 6 | 150 | 206 | 137 |
| | | 12.0 | 29 | 152 | 152 | 100 |
| | | 24.4 | 3 | 150 | 169 | 113 |
| Dead* | Males and females | 1.2 | 6 | 58 | 17 | 30 |
| | | 1.7 | 3 | 83 | 15 | 18 |
| | | 2.6 | 9 | 111 | 85 | 77 |
| | | 3.3 | 5 | 103 | 28 | 27 |
| | | 4.1 | 5 | 123 | 20 | 16 |
| | | 5.1 | 4 | 118 | 14 | 12 |
| | | 6.3 | 1 | 87 | 10 | 12 |
| | | 10.6 | 3 | 127 | 67 | 53 |
| | | 25.3 | 3 | 133 | 65 | 49 |

* Groups of dead aged less than 5.5 months include some pure ring doves.

years in the human. The figures found for the three kinds of pigeons therefore show a degree of thymic persistence that is perhaps relatively high for this age. Table 4 attempts to provide opportunity for a comparison of the pigeon thymus, at this and other ages, with the thymus of the human and the rat. These comparisons, however, are subject to qualifications which are discussed later.

One point concerning the early and rapid involution which immediately succeeds the attainment of maximum size may next be noted. As shown above, the maximum size occurs at 3.0 months and the period of very rapid involution extends only to about 6.0 months (see curves). This latter period certainly corresponds rather closely with the more usual time at which the females attain sexual maturity as indicated by egg-production. During this period of approximately three months the thymus of healthy common pigeons and ring doves lose about 50 per cent of their maximum weight. The more exact figures for this loss, as best obtained from the amount of thymus per 100 gram of body weight (tables 1, 2), are as follows: Common pigeon males (3.1 to 6.3 months), 55.5 per cent; females (3.0 to 6.6 months), 70.0 per cent; ring dove males (3.1 to 6.0 months), 37.5 per cent; females (3.3 to 5.9 months), 44.4 per cent. It thus appears that both the maximum growth and very much of the involution destined to occur during the whole life of the bird actually occur prior to the time of sexual maturity in the female.

Within the common pigeon group, and to a notably less extent in the other two groups, it is further apparent that the thymus of *adult* males is

larger than that of females. If, however, disease undiscoverable to us exists in greater degree in females than in males this result may be wholly or in part ascribable to such disease. Also, if the female thymus is more affected by confinement than is that of the male the observed difference may thus be partially or wholly accounted for. Of more definiteness and importance is the result that previous to the beginning of age involution the absolute size of the female thymus is fully equal to that of the male. The females moreover have a somewhat smaller body-size than the males and therefore apparently have a slightly larger proportion of thymic tissue

TABLE 4

*Proportion of thymus in body weight (body weight ÷ thymus) at various ages in man, rat and pigeon. (See text)*

| MAN | | COMMON PIGEON | | | | ALBINO RAT (FEMALE) | |
|---|---|---|---|---|---|---|---|
| | | Males | | Females | | | |
| Age | One part thymus in: | Age | One part thymus in: | Age | One part thymus in: | Age | One part thymus in: |
| | | *months* | | *months* | | *days* | |
| Birth | 240 | 1.0 | 287 | 0.9 | 389 | 7 | 385 |
| 5 years | 690 | 1.4 | 314 | 1.4 | 348 | 20 | 296 |
| | | | | 1.9 | 308 | 42 | 484 |
| | | 2.6 | 292 | 2.3 | 261 | 58 | 389 |
| 10 years | 960 | 3.1 | 289 | 3.0 | 273 | 70 | 445 |
| | | 3.7 | 332 | 3.8 | 351 | 85 | 502 |
| | | 5.4 | 479 | 5.4 | 639 | | |
| 15 years | 1140 | 6.3 | 648 | 6.6 | 914 | 113 | 810* |
| 20 years | 2356 | 8.1 | 494 | 8.0 | 1044 | 122 | 765* |
| | | | | | | 150 | 611 |
| | | 12.1 | 795 | 12.1 | 1209 | 162 | 1007 |
| | | 23.3 | 1267 | 24.5 | 1604 | 365 | 2183 |

* Figure is for males only.

during this period than have the males (see last column, tables 1 to 3). This result at this period (3.0 months) derives some further interest from the fact that in this case it is wholly improbable that confinement has at all influenced the thymus size, since up to the age of 1.5 months these young were in fact unable to utilize all the space actually afforded them; and from 1.5 months to near maturity they were provided with much more spacious quarters than during later periods.

If a curve be constructed from the data for common pigeons bearing *Ascaridia* it will be found essentially similar to that formed by the data

for the healthy birds. The most notable difference is found in the circumstance that thymus weights are there much lower during the first six months of life, and that the affected young females there do not have thymi equal to those of the males. In such a curve the size of the thymus of adult males is again nearly twice as large as that of the females. The difference in body weight of the adults of the two sexes is notable in both the healthy and *Ascaridia* groups, but only a small part of the observed difference in thymus size can be thus accounted for.

It should be noted that the presence of *Ascaridia* is not always associated with a small thymus. A small number of worms (1 to 5) is sometimes found in a bird with a normal or even an unusually large thymus. Larger numbers of these round-worms, however, are so commonly associated with a greatly reduced thymus that it seems necessary to give separate classification to birds found infested with these worms. That the thymus nearly or quite disappears in birds dead of disease, at all ages, is indicated by the data given in the tables. The tabulated averages fail, however, to reveal one fact made clear by the individual data, namely, that many birds dead of chronic disease often or usually have no discoverable trace of thymus. Further, the figures obtained for the various age-groups of dead birds are often unduly influenced by the inclusion of a single individual that died of an acute disease (after operation, peritonitis, etc.) and thus with practically a normal thymus. The experience, gained from the examination of the thymus in more than 2000 birds, convinces us that the size of the thymus more speedily and more often declares the presence of disease or of adverse conditions than does the macroscopic examination of any other organ of the bird's body.

The more scattering data obtained for thymus size in very advanced ages do not readily lend themselves to the construction of curves, or even to tabulation. Some data of this kind may next be considered. The weights in milligrams of the corresponding *thyroids* will here also be given (in parentheses, following the thymus weights). Table 1 shows that male common pigeons of 23.3 months have an average thymus weight of 296 mgm. Among the untabulated data for old healthy males are two thymi averaging 277 (thyroids, 87) mgm. from birds of 49.5 months; and two of 136 (99) mgm. from two very old birds of 84 months. Two female common pigeons at 77.5 months had thymi averaging 99 (46) mgm. The male ring dove hybrids at 23.5 months are shown in table 3 to have thymi averaging 144 mgm. Two of these at 43.2 months had thymi of 103 (20) mgm. Female rings aged 23.2 months show an average of 109 mgm. At 42 months figures of 93 (16) and 83 (19) were obtained from *Ascaridia*-infested birds, together with other thymi described as "medium" and "large" in birds aged 40 to 60 months. Table 2 shows that the thymus of male ring doves of 24.3 months weighed 90 mgm. An extremely old male (96.5 months,

with 3 *Ascaridia*) had a thymus of 55 (22) mgm. and a healthy female (94.3 months) a gland of 65 (21) mgm. Attention is here drawn to the fact that even in these birds of most extreme age, often not entirely free from disease, the amount of thymus present is 2 to 10 times the weight of the (usually enlarged) thyroids of these same birds.

In general the figures given above are for the largest of the few apparently healthy thymi weighed in birds of very advanced age. Three or four instances of markedly larger thymi than those mentioned were found among the entire lot of very old birds examined, but in these cases the organs were themselves obviously the seat of a tumor, or of calcareous deposit, and are here disregarded. Some further data may be given for another group of $F_1$ generic hybrids which often attain a most extraordinary age. The thymi of two males aged 136 months (body weight, 208 grams) average 90 mgm.; those of two females aged 132 months (weight, 188 grams) average 84 mgm. The figures already given, others that could be added of the type mentioned, and our more numerous descriptions (not weighings) of thymus-size in other old birds, all leave no doubt that a considerable amount of thymus tissue normally persists into the last stages of life in the kinds of birds studied by us. A small amount of data for one species (which does not well withstand captivity and confinement) suggests that even before old age its thymus is often much reduced. In such individuals, however, the gonads were also diminutive or reduced and breeding had been discontinued. It is probable that confinement was responsible, directly or indirectly, for these several changes.

Discussion. For the first time in a group of birds data have been obtained which adequately indicate the growth, and the time and extent of the age involution, of the thymus. These data show: that in the several kinds of pigeons examined the thymus normally persists in fair quantity in the oldest individuals; that at certain adult ages in our material (in common pigeons at least) the male thymus is notably larger than that of the female—but, prior to its involution, it is fully as large in the female as in the male; and that disease usually effects a pronounced or a practically complete reduction of the thymus in birds of all ages.

These facts seem in fair accord with the conclusions recently published by one of us (1) concerning a newly found function of the bird's thymus. It was stated that the data there given

> seem to demonstrate the presence in the thymus of a substance (for which the name *thymovidin* was proposed) having a highly specific action on the oviduct of birds—and presumably, of all those vertebrate animals which secrete egg-envelopes. The substance is indispensable to the production of normal egg envelopes. . . . . In this case we are probably dealing with the original or primary function of the gland.

In the bird species examined by us the thymus is normally present in amounts which suggest a continuance of its function throughout the whole reproductive period—even to the end of unusually long life. Again, the thymus is perhaps less intensely involuted in the old (healthy) bird—an animal in which this endocrine function of the gland is exercised—than it is in man and the rat at comparable ages. Apparently no great difference in this respect is indicated.

If the above-mentioned function of the thymus were its sole function, however, it would be very difficult to understand why any thymic involution whatever should occur either preceding or during adult stages—the latter being precisely the period during which egg-envelopes are actually produced. The very early and rapid growth, and the very early and rapid occurrence of most of its involution, would appear to be phenomena more closely related to other functions of the thymus. However these facts may be regarded, it is clear that in the pigeon, as elsewhere, this organ grows rapidly when the body as a whole is undergoing most rapid growth—though its rate of increase is for a short time apparently greater than is that of the body as a whole. Again, this period of rapid growth and of high proportion of body weight coincides with the period of complete immaturity of the gonads—particularly the testes. If it be true that the thymus exercises an influence upon either bodily growth or upon the development of the gonads, the abnormally large thymic development in very early life may be related to those facts. Relatively slight increase in body size occurs in pigeons after the beginning of the period which marks the beginning of size reduction in the thymus; and both ovary and testis accomplish their final stages of both growth and differentiation only after this same period. This type of relationship to the sex glands—if not a merely incidental and quite fortuitious accompaniment of the development of the organism—would here again involve a relation of the thymus to both sex and reproduction. A closer examination of the relationship of the curve for thymic growth and involution to the growth and functioning of the gonads will be made in a later study.

The unexpectedly early occurrence of age involution of the thymus in pigeons (and the probable presence of unrecognized disease or of adverse conditions in the few youngest birds examined) apparently caused McCarrison to overlook it entirely. It seems fairly clear that his examination was made essentially upon thymi which had already undergone the early and rapid stage of age involution; probably it was a failure to find pronounced size changes at (perhaps) 5 or 6 months that led to his conclusion that "in so far as pigeons are concerned, the involution of the thymus is dependent on factors other than maturation of the sexual function; these factors are mainly nutritional." The present more extensive data show that the chief point in the independence of involution and maturity

lies in the very early occurrence of the former; and further, that nutritional factors can perhaps explain the character of no part or point of the normal thymus curve, though McCarrison's data prove that the temporary involution of the gland can at all times be effected through nutritional factors. We have sectioned very few thymus preparations. Those, however, agree with conditions described by McCarrison, and quoted earlier in this paper. Our data also fully agree with his conclusions concerning the effects of disease, and in large measure confirm in adult (only) common pigeons a sex difference in thymus size. If such a sex difference exists in the two other kinds of pigeons studied it is probably a less notable difference.

Jolly's few observations in the fowl would apparently indicate that age involution here occurs at or after 5 months. The data, however, are acknowledged to be quite insufficient, and the point still requires a further and thorough study. From his own data Jolly states that it seems clear that in the fowl "the thymus attains its maximum weight and development, and presents no sign of involution, at the precise moment (5 months) of the attainment of sexual maturity and the appearance of sperm in the genital tract." If this point can be fully confirmed in several individual fowls we should thus obtain a fact of importance. Nothing is said of the condition of the thymus at the time of sexual maturity in the female fowls; the breed of fowls used is not stated, and any estimate of this point is thus excluded.

The possible effects of season and of confinement require a brief consideration. As noted earlier in this paper, Jolly lists "seasonal changes" as having an influence on thymus size. No supporting data are cited. Aimé's observations on the turtle, and our own casual observations on wild birds shot at periods outside the breeding season, at least suggest the truth of this view. We hope to obtain further data on this point. Certainly the probability as stated must be taken largely into account in any observations made on wild forms; and cold weather, or another kind of weather, may prove to be merely another "adverse condition" which is immediately followed by a temporary reduction of thymus size. It is certain that the gonads—particularly the testes—of these wild birds undergo enormous reduction immediately following the close of the breeding season.

Confinement may or may not affect thymus size. It has been definitely learned in this laboratory, however, that confinement does lead to diminished testis size in some wild species of pigeons, and it has long been known that it tends to suppress reproduction in both sexes. It is further known that very close confinement (10) may check reproduction even in domesticated pigeons; and it is certainly true that a degree of confinement which is little or not at all "adverse" for a domesticated race is usually plainly so for a wild species. For these reasons references to and data concerning the degree of confinement of our material have been given in this communication.

The source of the data for the comparisons of table 4 should be stated. Those for man are quoted from Paton (6) who does not cite the original work. For the rat the data are those of Jackson (11) and Hatai (12). In fixing comparable age-groups of the human and the pigeon the well-known facts for man, and our own data for pigeons, are utilized. Comparisons of the pigeon and rat also make use of Donaldson's (13) findings on the time of maturity and length of life of the albino rat. Donaldson states that these rats "usually begin to breed at 90–100 days," and that "sexual maturity as indicated by the structure of the gonads may occur at 60–70 days." Further, that some rats "may live to an age of 40 months," and the extreme age observed was 45 months.

In the data obtained for the rat the female thymus was slightly larger (perhaps insignificantly), according to Hatai, than that of the male. In our table the data for female rats are used in those age groups for which such data are available. It does not appear that diseased rats were entirely excluded in the data of Jackson and Hatai, though it is stated at one point that "the animals were in good condition." Whether healthy human material formed the basis for the calculations which we quote from Paton may be doubted. If so, it is clear that the comparative figures obtained for both the human and rat, relative to the pigeon, may at all ages really show too small a proportion of thymus in those mammals. It is quite possible that the data for neither two of the three species may properly be compared. The desirability of a proper comparison is evident; we have made such comparison as is now possible.

Data similar to those given here for pigeons are much needed for other kinds of birds. Certainly the facts concerning thymic growth and involution will be adequately known for birds only when several species including both domesticated and wild forms have been examined. Such data for reptiles, amphibians and fishes are also much needed. If the thymus ever completely and normally disappears in any of these groups, and if any of these forms should prove capable of normal reproduction long after the complete absence of the thymus, the relationship found in pigeons between the thymus and the production of egg-envelopes would become of limited or questionable application. The first apparently adequate test of a few species of one of these groups, as presented in this paper, shows that such limitations have not been encountered in pigeons. The differences that may exist between the conditions found in the above-named classes of vertebrates on the one hand, and of mammals on the other, will serve to test the view earlier stated by Riddle; namely, that, along with the changed method of reproduction under which egg-envelopes are no longer produced by mammals this class of vertebrates has lost one, and probably the primary, function of the thymus.

SUMMARY

A true age involution occurs in apparently all species and races of pigeons.

The relationship of disease and other adverse conditions to thymus size in various pigeons of all ages is considered. It is found that the thymus, through its size reduction, more speedily and more often declares the presence of disease or adverse conditions than does the macroscopic observation of any other organ.

Curves for the growth and age involution of the thymus are given for apparently healthy individuals of three kinds of doves and pigeons. These curves are the first obtained for any species of birds, and the results have a bearing on the question of the incretory (*thymovidin*) regulation of egg-envelope production in non-mammalian vertebrates.

Age involution occurs at 3.0 months (2.5 months after hatching), which is—at least in the female—notably earlier than the attainment of sexual maturity as measured by the beginning of egg laying.

Within the period of three months extending from 3.0 to 6.0 months, that is, from the time of maximum thymus development to the time females usually attain sexual maturity, the thymus decreases in weight in both sexes by approximately 50 per cent.

Advanced age in pigeons is accompanied by a further progressive decrease in the weight of the thymus; but in normal healthy birds of extreme old age the thymus exists in amounts always far in excess of the amount of thyroid tissue present.

Before age involution begins the female thymus is fully as large as that of the male. During periods (8 to 24 months) which immediately succeed the attainment of sexual maturity, however, the thymus of male common pigeons is notably larger than that of the female; this part of our results confirms the observations of McCarrison.

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# THE EFFECTS OF CEREBRAL DESTRUCTION ON THE SEXUAL BEHAVIOR OF RABBITS

## I. The Olfactory Bulbs[1]

CALVIN P. STONE

*From the Department of Psychology, Stanford University*

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The purpose of this investigation was to determine whether olfactory stimuli are necessary for the activation of congenital sexual responses in rabbits. This information is needed in order to determine the feasibility of combining in one animal the destruction of olfactory bulbs and various portions of the cerebral cortex, thereby, having eliminated olfactory impulses from the Rhinencephalon which by virtue of its position in the calvarium is largely inaccessible for ablation, rendering the cerebrum more quiescent than has heretofore been accomplished in animals by the destruction of accessible portions of the cortex alone.

According to views ofttimes repeated in the literature, the olfactory sense plays an important rôle in the activation of sexual behavior in animals. Two of the older hand-books of physiology (5, 7) state that the sense of smell exercises a special influence on the sexual life and that for many animals it is the weightiest although not the only factor activating the reproductive instincts.

More recent investigators writing on the physiology of sex have, for the most part, followed the lead of the earlier physiologists in this matter. Von Bechtrew (1) in a discussion of the sexual instincts says that in the higher animals the olfactory sense stands in close relationship to the sexual functions; for many it is a strong activator of the pairing impulse. Steinach (9) similarly emphasises the rôle of olfaction in the arousal of sexual responses at a time when the nervous system has been prepared for sexual activity through the influence of secretions derived from the gonads.

According to Havelock Ellis (2), who defines the exteroceptive stimuli most potent for arousing and heightening the sexual impulses, olfactory stimuli occupy the chief place among the lower animals. Even in man the olfactory sense is secondary only to touch.

[1] Financed jointly by funds from the Thomas Welton Stanford Endowment for Psychological Research and the National Research Council through its Committee for Research on Sex Problems.

Notwithstanding the fact that little if any substantial experimental evidence has been brought forward to support these views, their validity has seldom been questioned. To some writers, no doubt, their truth is self evident and therefore need not be supported by controlled experiments. Indeed, anyone needs hardly go beyond the range of casual observations to find what appears to be supporting evidence. Various amounts of "sniffing" almost invariably precede the reproductive act of a great variety of domestic and laboratory animals. Post hoc, ergo propter hoc; and without further investigation an uncritical observer assigns to the sense of smell the fundamental rôle of activator of the sexual impulses.

A moment's reflection suffices to dissuade one from the belief that the rôle of olfactory stimuli in the activation of sexual bahavior can be evaluated from such superficial observations. "Sniffing," so-called, in connection with the reproductive act sometimes involves a considerable amount of cutaneous stimulation not only of the animal sniffed but also of the sniffer, himself. These cutaneous stimuli applied to the external genitalia (erogenous zones of the female) may, and frequently do, evoke patterns of response specific for the animal species and characteristic of the phase of the oestrual cycle in which the animal at that time is found. Obviously, then, when a variety of stimuli are acting upon the male in full possession of his sensory equipment, it is next to impossible to determine the particular kinds of stimuli most effective in activating the sexual responses. On the other hand, by observing the behavior of a male that has been deprived of the sense organ through which any kind of stimulus in which we are interested is normally mediated, one may arrive at some conclusion as to the importance or the necessity of this stimulus as an activator of sexual behavior. To illustrate, if the sexual responses are elicitable after the male has been deprived of the sense of smell one is justified in concluding that the olfactory stimuli are not a sine qua non for the activation of sexual behavior. Furthermore when, in the absence of olfactory receptors, there is no observable handicap in the initiation of sexual responses either in the young or the fully developed adult one may conservatively conclude that olfactory stimuli are not to be looked upon as the prime movers in the activation of sexual activity. If at all serviceable in arousing or heightening the sexual impulses other stimuli in their absence may readily be substituted for them.

In the case of certain animals there can be little doubt that the sense of smell is unnecessary and probably unimportant for the arousal of sexual behavior. Fowls as a group depend little if at all upon the sense of smell either in life begetting or life supporting activities. In them the olfactory regions of the brain are poorly developed as contrasted with most other vertebrates both of lower and higher rank. Furthermore the few investigations of the breeding activities of vertebrates rendered anosmic by the

destruction of the olfactory bulbs show that the sense of smell can be dispensed with in the animals studied without marked effects on their sexual behavior.

According to Luciana (6), olfactory stimuli are not essential for the sexual reflexes of frogs and toads. He says, "Removal of the most sensitive parts and of the whole brain, including of course the olfactory and visual organs, does not inhibit the sexual clasp nor interrupt it if already in progress." Golz (4) has shown that all accessible special sense organs can be successively removed from different male frogs without their ceasing to copulate with the female.

Von Bechtrew (1) observed the sexual behavior of dogs with bilateral destruction of the olfactory bulbs and concluded that the "libido sexualis" was unaffected. The only noticeable difference in behavior of an anosmic and a normal dog was a greater tendency on the part of the former to indiscriminate mounting of other dogs. A rather extensive series of observations on blind and anosmic white rats previously reported by Stone (10) led to the following conclusions concerning the activation of sexual responses in the male: The elimination of visual, olfactory and gustatory sense functions in young males prior to sexual experience and in sexually experienced adults produces no changes in the sexual act, either in the essential pattern, the age at which it appears, or the responsiveness of a male to a receptive female.

On the positive side, it may be said that, so far as the author has been able to ascertain, there is no well-established case of a vertebrate animal that depends primarily upon the sense of smell for the activation of sexual responses.

TECHNIQUE, THIS EXPERIMENT. *1. Diets of animals.* The dietary factor is known to be a potent agent for modifying, retarding, and suppressing the sexual behavior of laboratory animals. (See especially the work of Evans (3) and Slonaker (8).) Accordingly, special attention was given to the feeding of animals before and during experimentation. An ample supply of fresh water and what rabbit breeders of this locality believed to be a well-balanced food ration was provided. The constituent elements of the diet provided were as follows:

Alfalfa hay, ad libitum
Rolled barley approximately 150 to 300 grams fed each morning and night to an adult or half-grown rabbit
Cabbage and carrots once or twice per week.

The foregoing diets appear to be adequate for the normal growth and reproduction of rabbits. On this they thrive and reproduce throughout the year.

*2. Operative technique.* The technique followed in transecting the olfactory bulbs of rabbits was as follows. The hair was removed from the operative field and the skin thoroughly saturated with iodine. With animals under deep anesthesia an incision was made in the scalp at a point midway between the eyes. Thence it was carried nasalward one-half inch and backward approximately one-half inch. The skin on either side of the incision was retracted and raised from the skull plate. With fine bone forceps an opening in the roof of the skull was made at the midpoint of a line connecting the posterior border of the eyes and extended backward about 2 mm. Through this opening a cautery knife heated to redness was inserted and the olfactory bulbs completely transected at the anterior border of the frontal poles. In some cases a small portion of the most proximal part of the frontal poles was unintentionally removed when the olfactory bulbs were transected. After all bleeding had ceased the wound was cleansed with sterile cotton and closed by bringing together and suturing the edges of the skin over the opening in the skull. A thick coat of celloidin was then placed over the incision to protect it during the time required for healing.

Post-operative effects were relatively mild. As a general rule the animals resumed eating on the same or the day following the operation. During the first week some loss of weight was recorded for all, but no permanent retardation of growth was observed even in the younger animals. Two weeks after the operation post-operative effects could not be discerned in the cage activities of the animals.

At the close of each experiment the animals were sacrificed and the brain examined to determined the extent of the lesion. All animals herein reported had completely transected olfactory bulbs with little or no destruction of the frontal lobes of the brain.

RESULTS FROM EXPERIMENTS ON RABBITS. 1. Operations performed prior to puberty: *Cases 1 and 2:* Flemish giants, reared apart from all females from the age of 45 days. Olfactory bulbs transected at the age of 77 days. Forty-four days later (animals' age 121 days) a receptive female was placed into their cage. Without delay each young male gave attention to the female and within a few minutes without any preliminary sniffing in the region of the external genitalia, each had mounted and copulated with this female. In due time the female bore a litter begotten by one or both of these males. Approximately one month after the males had demonstrated sexual activity, they were sacrificed. Necropsy revealed complete transection of the olfactory bulbs.

*Case 3:* Flemish giant, reared apart from females from the age of 45 to 121 days. Olfactory bulbs severed at the age of 77 days. When the male had attained the age of 121 days a receptive female was placed into his cage. Breeding ensued within a few minutes with only a slight amount of nosing in the region of the female's external genitalia. Female conceived and delivered a litter of nine young. Necropsy showed complete transection of the olfactory bulbs.

*Case 4:* Flemish giant, reared with males only from the age of 45 to 121 days. Operated at the age of 77 days. At the age of 121 days a receptive female was placed into his cage. No attention to the female was given; the latter, however, gave attention to the male as manifested by her repeated attempts at mounting him and going through the pelvic movements of the male performing the reproductive act. The female was left with this male until evidence of her impregnation was at hand. Allowing 30 days for the period of gestation, the age of the male at the time the female was fertilized was 159 days—an age that is probably within the normal range of variability for sexual maturity, according to advice from local breeders. The cause of this male's failure to impregnate the female at an earlier date cannot be given. Observations of his behavior in the presence of the female at various times when she was sexually receptive, however, led the experimenter to believe that the fault lay with him alone. In his case it is clearly evident that the awakening of sexual behavior was somewhat retarded as compared with other animals employed in this study, but at the present stage of the investigation the cause cannot be definitely assigned. Retardation may have resulted from the deprivation of the olfactory stimulation although no evidence of similar retardation was apparent in the four other young males similarly operated. On the other hand it may have resulted from operative shock or a nutritional disturbance not directly manifested in the behavior or the growth of the male. Necropsy revealed complete destruction of the olfactory bulbs without injury to the frontal poles.

2. Operation performed on the adult: Two adult Flemish giants of unknown age. Had been in the psychological laboratory for approximately one year and when purchased from a local dealer were called young adults. Twenty-four hours after the olfactory bulbs were transected each male was placed with a receptive female. Within five minutes copulation was observed. Although these males had formerly sniffed or nosed females preliminary to copulation, this behavior was now absent from pre-copulatory activity. No handicap to sexual activity was observed either immediately after the operations or during the month subsequent thereto. They retained their usual sexual vigor and attempted copulation with females put into their cages irrespective of their receptivity as had been their habits prior to operation. Necropsy one month after the operation showed complete transection of the olfactory bulbs.

## SUMMARY

From the foregoing experiments it becomes apparent that the loss of the olfactory bulbs either in the young or the adult has no serious effects on his ability to enter into sexual activity. If the sense of smell is used in connection with sexual behavior at all, in its absence other senses take over its function with so little handicap to the animal that no significant alteration of his behavior is perceptible to the observer. Subsequent experiments have shown the feasibility of combining the destruction of olfactory bulbs andvarious portions of the cerebral cortex in a single animal for further study of the relations of the cerebral cortex to congenital sexual behavior.

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# THE INTERRUPTION OF PREGNANCY BY OVARIECTOMY IN THE APLACENTAL OPOSSUM: A STUDY IN THE PHYSIOLOGY OF IMPLANTATION[1]

CARL HARTMAN

*From the Department of Zoölogy, The University of Texas, Austin, Contribution No. 187*

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HISTORICAL. The influence of the ovary on the development and cyclic activity of the uterus has long been established.[2] Since Prenant (1898) called attention to the glandular nature of the corpus luteum and Born about the same time postulated his theory of implantation, the corpus luteum has received almost exclusive attention of experiments in this field. Fränkel was the first to furnish the experimental basis for Born's hypothesis, which has since been numerously corroborated and is, in principle at least, generally accepted.

The Born-Fränkel theory may be stated as follows: The corpus luteum is a gland of internal secretion. It effects those changes in the uterus which make possible the implantation of the egg (9). The experimental evidence may be classified under these heads:

1. Double ovariectomy[3] practised on rabbits during the first half of the period of gestation results in the death of the embryo.

2. Identical results are attained by the destruction of the corpora lutea.

3. Cauterization of ovarian tissue around the corpora lutea, these being left intact, does not result in death and resorption of the embryos. Such control experiments effectively answer Schauta's objections which are to the effect that the trauma incident to extirpation of the corpora lutea inhibits the function of the ovary as a whole.

4. In cases of unilateral ovulation and therefore the formation of corpora lutea in but one ovary, removal of this, leaving the other intact

[1] The experiments here recorded extended over a series of years. They were begun under the financial and moral encouragement of the Wistar Institute of Anatomy and Biology, Philadelphia, Dr. M. J. Greenman, Director, and were continued with the aid of grants from the Bache Fund of the National Academy of Sciences, and from Mr. H. A. Wroe, member of the Board of Regents of the University of Texas.

[2] The present state of our knowledge concerning the interrelation of the female reproductive organs has recently been summarized by Evans (7), Marshall (33) and Corner (5).

[3] The term *ovariectomy*, although also a hybrid word, seems preferable to the incorrect term *ovariotomy* as well as the correct but cumbersome *oöphorectomy*.

(Mandl's method), results in the death of the embryo (7 cases, Fränkel, 1910, p. 21). These experiments gainsay Mandl's results (31) and answer his argument for the action of the "ovary as a whole."[4]

Fränkel's chief criterion of corpus luteum insufficiency was the death and resorption of the embryos. But unlike some other workers he adds full details in his protocols concerning the behavior of the uterus in the experimental animals. In many respects his descriptions fit those of the opossum uterus under similar conditions, as will appear below.

Fränkel (8), (9) dealt chiefly with the gravid uteri of rabbits and made no attempt to examine the effect of the corpora lutea on the non-gravid organs during the stages of the oestrous cycle. This was done by L. Loeb (23), (24), (25), (26), (27) who worked on the guinea pig, which ovulates spontaneously, and on rabbits in which he prevented pregnancy by ligation of the fallopian tubes after coitus. Further observations were made by Bouin and Ancel (30), who varied the method of Loeb by inducing ovulation but preventing pregnancy in female rabbits by mating them with vasectomized males.

The French authors established the identity of the corpus luteum of ovulation and the corpus luteum of pregnancy; and in correlation with the growth of the former, contrary to the findings of Regaud and Dubreuil (39), observed in the non-pregnant uterus progressive, pregravid growth, proliferation, hyperemia, and an infiltration of lymph into the connective tissue spaces of the mucosa consequent upon the vaso-dilatation of the capillaries. After the fourteenth day both corpora lutea and uteri were found to involute.

In the meantime L. Loeb had discovered a specific, clearly demonstrable effect of the corpora lutea, namely, the "decidual reaction" of the uterine mucosa. This he established by a wealth of experiments (1907–1917).[5] The phenomenon constitutes one of the most beautiful demonstrations of the effect of hormones in the field of endocrinology. The facts are that in the rabbit from the second to the ninth day of development the corpus luteum gives off a secretion that sensitizes the uterine mucosa so that any mechanical stimulus is effective in causing masses of decidual cells to develop (deciduomata) which closely resemble those found in the early stage

[4] The well-considered experiments of Mandl suggest the desirability of testing his contention in the case of uniparous animals in which usually only one of the ovaries contains a corpus luteum of a given gestation. Since our small laboratory animals are all multiparous and our larger domestic animals too valuable, monkeys and certain Australian marsupials would seem to be the best adapted for these experiments.

Note : Since writing the above I find that Wester (53) records a dozen appropriate experiments on pregnant cows (pp. 36 and 37) with results concordant with Fränkel's contentions.

[5] Loeb, L. (27) contains a summary of his brilliant results together with a fairly complete bibliography.

of normal pregnancy, in which, presumably, the fertilized ovum offers the adequate stimulus.

Loeb's findings were confirmed by Gasbarrini (12), by Corner and Warren (6), and by Nielsen (37) on rabbits and by Frank (11) and Long and Evans (29) on rats. The reaction has found further confirmation in the experiments of Biedl, Peters and Hofstätter (2). These workers attempted further to answer two questions: 1, are the corpora lutea indispensable for the placental reaction? and 2, is this reaction necessary for implantation? The method employed was that of Heape (18), namely, the transplantation of fertilized rabbit ova into foster mothers which at the time possessed no corpora lutea. The authors express the belief, 1, that they noted decidual cells in six such cases; and 2, that in one case the embryo actually became implanted. On the whole, the results of these ingenious experiments may be said to be suggestive but inconclusive.

The reader will note that the experiments cited above concern the laboratory rodents only, in which the decidua develops to a high degree of perfection.[6] In view of the importance which gynecologists attach to the phenomenon it seems the more remarkable that the experiments of Loeb and of Fränkel have not been extended to other orders in which there are different types of implantation. The dog seems to be the only other form studied. Marshall and Jolly (34) promptly confirmed Fränkel by showing that castration of bitches early in pregnancy caused the death of the embryo and their resorption in utero. Krainz (22), also working on dogs, found a placenta-like hypertrophy of the uterine mucosa adjoining incisions into the uterine horns, but he also noticed that mechanical stimulation by foreign bodies introduced into the uterine horns *fails* to elicit any response on the part of the endometrium.

It is thus apparent that the decidual reaction of Loeb has overshadowed every other process that takes place in the uterus of early pregnancy, such as increased vascularity, lymph secretion, hyperplasia of the glands and tonus of the musculature, to which Fränkel (8), (9), Bouin and Ancel (3), Hitschmann and Adler (19), Schröder (47) and many others have called attention. That the wrong emphasis has been placed upon the decidual reaction the following experiments performed upon the opossum would seem to indicate. For in this animal, that has no placenta, hence no decidual cells at any time, it ought to be possible, if the decidual reaction is the *sine qua non* of implantation, to remove the ovaries with impunity during pregnancy. Such a conclusion seems reasonable on *a priori* grounds because birth takes place but five days after the corresponding stage is

[6] An important hiatus in our knowledge of the processes under discussion is a satisfactory account of the comparative anatomy of the decidual cells in mammals, as suggested by Ritter (40), who made a beginning in this direction. See also Grosser's book (13).

reached (primitive streak) at which in the rabbit implantation normally takes place.

The experiments detailed below prove, however, that in the opossum the ovary is as necessary for the nutrition of the embryo as in the rabbit. The problem of the relation of the ovary to implantation is, therefore, by no means settled and must be further investigated before the underlying factors are understood. The decidual reaction, clear and definite as it seems, is, where it occurs, probably only a link in a chain of processes. No one link is more important or essential than another.

The results of our own experiments will next be summarized. For additional details the reader is referred to the protocols printed at the end of the paper.

Results. The foregoing experiments concern 33 ovariectomies performed upon female opossums in various periods of the oestrous cycle or of pregnancy. The results will be presented under several heads according to the state of the animals at the time of operation.

*1. Animals operated in anoestrum.* Nos. 154 and 155 were adults operated during the period of sexual rest and no. 181 was an immature female. None of these animals showed any signs of sexual activity after castration. This is in accordance with the results of castration in the higher mammals.

Other cases in which the operated animals were kept a month or more (nos. 555 and 889) returned to the anoestrous condition (fig. 6).

*2. Animals operated in early pseudopregnancy.* No. 583 died two days, no. 743 three to four days after the operation. In both of these some retrogressive changes were apparent, especially in the latter. No. 554 was killed 6 days; no 605, 7 days; no 804, 8 days; no 594, 9 days after the operation. In all of these cases retrogressive changes were marked as compared with the normal pseudopregnant uterus which at about eight days after ovulation is indistinguishable from the pregnant organ (16), (17). The degree of involution depends on the individual resistance as well as the length of time that elapses after extirpation of the ovaries. Thus the uterus of no. 804 showed great degeneration (fig. 5); no. 605 (fig. 9) less than no. 804, but greater than is ever seen in the normal metoestrous organ.

*3. Animals operated in late pseudopregnancy.* Nos. 237, 239, 241, 248 and 252 were completely castrated and, in order to determine the age of the ova, one uterus was removed at the time of the operation. Other control females in comparable stages were also hemi-hysterectomized but only semi-spayed. Comparison of the two series showed a more rapid retrogression of the surviving uterus in the double ovariectomized females.

*4. Animals operated in late pregnancy.* No. 813 died 4 days after the operation; some degeneration was noticeable in the uterus. In the case of no. 186, the operation brought on an abortion, which sometimes occurs

also in semi-spayed females operated near term. A 2-day interval witnessed no perceptible change in the uterus of no. 616 and only a slight change in no. 573 in which the uterus had assumed the peculiar elongated form characteristic of the uteri in this series of experiments. But the operation affected greatly both the uterus and its contents in the case of no. 829 and still more so in the case of no. 795. The uterus of the latter 5 days after the operation presented exteriorly the usual picture of the uterus in castrated animals, but the organ constituted a veritable sack of pus, for the mucosa was profoundly degenerated and infiltrated with clouds of bacteria (fig. 10). The embryos had been almost completely resorbed.

*5. Animals spayed in early pregnancy.* This constitutes the most instructive group. Three animals were castrated when the fertilized eggs were in the cleavage stage (nos. 602, 794, 925); three had very young blastocysts (nos. 604, 927 and 582), one had bilaminar blastocysts (no. 581) and only one had vesicles that had advanced to the primitive streak stage (no. 580). It should be noted that the latter eggs would normally have attained the stage of parturition about six days later; yet in 4 days, 19 hours after ovariectomy the embryos had died and were partially resorbed (fig. 7). In the case of no. 582 a three-day interval after castration witnessed no noticeable effect. Animal 581 died; but the uterus in the three-day interval showed the typical collapse following ovariectomy. No. 927 proved the most resistant, for six days after the operation two embryos were still apparently normal though the uterus was palpably defective. In all the other experiments (nos. 580, 602, 604, 794 and 925) the uteri had undergone the typical collapse by absorption of lymph from the mucosa, and were in various stages of involution (figs. 1 and 2). It is significant that embryos of considerable size with their envelopes had developed in all cases but one (no. 925) before death and resorption overtook them.

Without exception, then, ovariectomy performed soon after ovulation results in regressive changes in the uterus which in pregnant animals prove to be prejudicial to the life of the embryos. The first change apparent is the resorption of lymph from the central layer of the mucosa. For normally, in the pregnant or pseudopregnant opossum uterus, this layer is tremendously dilated with lymph in which the coiled glands and the blood vessels are suspended. The chorionic membranes of the embryos are closely applied to the uterine epithelium and follow this as parallel sheets throughout its complicated folds (fig. 8). It is at these two membranes that the maternal and the fetal circulations come into close relation (see 17, fig. 26).[7] This relation is disturbed upon the collapse of central layer of the mucosa (fig. 7) and the embryos languish for lack of the nutritive exchange.

[7] A similar quite simple relation of chorion and uterine mucosa is found in the pig, in which the relation of the ovary to implantation should likewise be investigated.

The resorption of lymph results in a loss of turgidity to the organ as a whole, already manifested from gross inspection. The normal turgid organ possesses a brilliant glass-like luster like polished red agate ("rotglasern," Fränkel). In such an organ an incision of a centimeter through the musculature results in a hernia through which the embryos and their envelopes are ejected one by one, as in figure 11. The internal pressure may be referable to the tonus of the musculature (cf. 17a) as well as to the fluid content.

All this is changed in the flaccid organ, which becomes dull, elongates and may easily be opened by a longitudinal slit without disarranging the contents of the organ (cf. figs. 7 and 11).

*6. Controls.* During the course of some years' work on the opossum, one ovary and the corresponding uterus were removed in scores of cases. The operation had no noticeable effect on the course of gestation in the surviving uterus. One ovary was found sufficient to maintain normal conditions in the surviving uterus. This matter I have already fully discussed (15, pp. 25 to 29).

In three of the present series of experiments, however, one ovary and one uterus were removed from *contralateral* sides. For it seemed desirable to determine whether ligation of the ovarian artery and section of the mesovarian had any deleterious effect on the corresponding uterus. It was found that these animals, nos. 585, 931 and 841, had perfectly normal uteri 5, 6 and 8½ days respectively after the operation. The uterus of no. 841, for example, shown in figure 3, was found to be large and turgid exactly as it would have been without operative interference (cf. figs. 3 and 1). This is true despite the fact that the surviving ovary was adhered to the adnexa (*ov.*, fig. 3). A section through this uterus with a normal embryo is shown in figure 8. The chorion may be seen closely paralleling the epithelium of the uterus. This is distended with lymph in which the glands are seen to float.

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Note: The reader is referred to the protocols for additional details concerning the animals.

Fig. 1. Genital tract with surviving right uterus of animal no. 602, 8 days after double ovariectomy. × 1.

Fig. 1a. The ova removed from the left uterus at the time of operation. The eggs are in the 16-celled stage. × 8.

Fig. 2. The same as figure 1, with the flaccid uterus laid open, showing remains of macerated embryos. × 1.

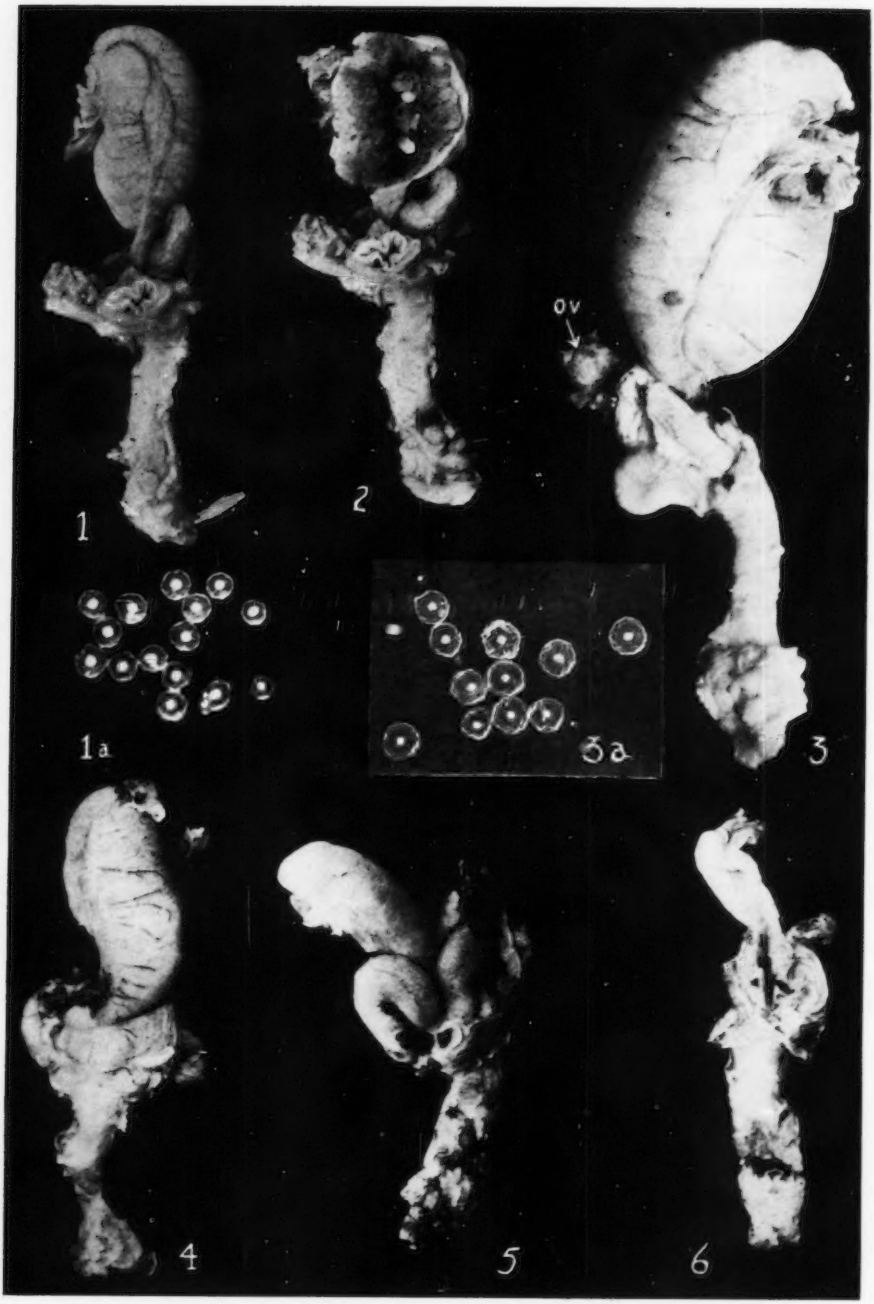
Fig. 3. Control experiment, animal 841, 8½ days after the operation. The normal large and turgid left uterus is shown. *Ov.*, surviving right ovary. × 1.

Fig. 3a. Ova removed from right uterus of no. 841 at time of operation. The ova are in about the 16-celled stage. × 8.

Fig. 4. Genital tract of animal 794, 8 days after double ovariectomy. × 1.

Fig. 5. Genital tract of no. 594, 9 days after double ovariectomy. × 1.

Fig. 6. Genital tract of no. 555, one month after double ovariectomy. × 1.


ov
1
2
3
1a
3a
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6

In the three special controls, as in scores of others, the surviving ovary maintained the normal turgidity of the uterus, in striking contrast to the condition in which both ovaries are removed.

Such are the facts. Their explanation is another matter. Some of the more general bearings of the observations recorded will now be discussed.

DISCUSSION. 1. The outstanding feature of the preceding experiments is the death of the embryos after double ovariectomy. The ovary is shown to be necessary for the continuance of pregnancy in the opossum, which is thus brought into line with the higher mammals in this regard. The work of Fränkel, L. Loeb, and of Bouin and Ancel on the corpus luteum in its relation to the uterus and to implantation makes it seem highly probable that the interruption of gestation in my experiments is referable to the ablation of the corpora lutea.[8]

2. The experiments here recorded on the pregnant female opossum agree strikingly with the results of Fränkel on the rabbit. My experiments are also open to the criticism of Loeb (27), leveled at Fränkel's experiments, that "pregnancy is readily influenced by other operative and experimental procedures" (27, p. 11). But Fränkel's numerous controls speak for themselves and seem to me quite sufficient. As for the opossum, I have performed scores of laparotomies and unilateral ablation of ovary and uterus without injury to the surviving uterus or its contents. Furthermore, the three cases recorded above, in which I removed one uterus and

[8] The opossum ovary in the pregnant animal often consists of little else than corpora lutea. The interstitial tissue, while occasionally present in large amounts, is often almost entirely absent, especially at the beginning of the breeding season. Loeb and Marshall and Jolly speak in similar terms of the ovary of the guinea pig.

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Fig. 7. Section through wall of uterus and macerated embryo of animal 580 nearly 5 days after double ovariectomy. *AM*, amnion; *CH*, chorion and uterine epithelium; *B*, basal layer of mucosa with normally involuting glands; *M*, muscle layers. The central layer of the mucosa is collapsed (compare *C* of fig. 8). × 8.

Fig. 8. Section through uterus and normal embryo of control animal 585 (cf. fig. 3) 5 days after removal of left uterus and right ovary. The normal, greatly swollen central layer of the mucosa, *C*, with the coiled glands floating in lymph is in sharp contrast to the collapsed condition shown in the preceding figure. × 8.

Fig. 9. Section through greatly involuted uterus of pseudopregnant animal 605, 7 days after double ovariectomy. Letters as in figure 7. × 8.

Fig. 10. Portion of section of uterine mucosa of no. 795, showing atrophic glands. The dots are polymorphonuclear leucocytes. × 50.

Fig. 11. Normal turgid uterus of no. 573. A slit in the muscularis caused a hernia through which several embryos and their envelopes have been protruded. × 1.

Fig. 12. Degenerating embryo from the uterus of no. 794, 8 days after double ovariectomy. *AL*, allantois, which continued growing after embryo had died.


AM
SH
B
M
B
7
CH
C
B
M
8
M
9
10
11
AL
12

the contralateral ovary without in the slighest affecting the surviving uterus fully justify Fränkel's method as applied to the opossum.[9]

3. In consonance with this criticism Loeb demanded that the relation of uterus and ovary be studied in non-pregnant animals, in which the uterus is observed "without the interference of the fertilized ovum." Carrying out this idea Loeb discovered the "decidual reaction" of the endometrium briefly reviewed above. Bouin and Ancel (3) also studied the influence of the corpus luteum on the non-pregnant uterus by a variation of Loeb's method and described growth changes and other conditions of the uterus after ovulation and the development of corpora lutea.

The opossum is particularly well adapted for such observations because *1*, ovulation is spontaneous, and *2*, the uteri develop, in correlation with the high develpment of the *corpora lutea ovulationis*, a stage of pseudopregnancy unknown in the higher mammals. With ablation of the ovaries a certain considerable development is nevertheless attained, the growth process having been well under way at oestrus (17); but spaying causes a more rapid and sometimes a more profound involution as compared with the normal. The pseudopregnant opossum uterus shows the same sudden collapse of the mucosa due to resorption of lymph exhibited by the pregnant organ.

Loeb's demand is thus adequately complied with.

4. One of the first noticeable effects of the elimination of ovarian function is the peculiar elongation of the uterus. One gets the impression that longitudinal muscle fibers were the first to be inhibited in their tonus.[10] The organ then becomes flaccid. This lack of internal pressure is probably due both to the relaxation of the muscle fibers and to the resorption of lymph from the formerly greatly hypertrophied mucosa. In shape and consistency the organ is like the post-partum uterus; but whereas this is of a red-purple color, the former is pale and yellowish.

In all these points the opossum and the rabbit agree, as judged by Fränkel's protocols, as well as by the descriptions of Bouin and Ancel.

[9] Pregnant animals injured in steel traps or badly torn by dogs are often brought into the laboratory. Occasionally the embryos are all dead, due to direct trauma of the uterus; but usually the embryos seem none the worse for the mother's experience. Such injured animals, however, usually fail to come into heat again. The ovaries, I find, are much more sensitive to harmful stimuli than the pregnant uterus. For these reasons, as well as clinical experience generally, I an convinced that Loeb and Hunter's conclusions are not fully justified. Their conclusions are, moreover, based largely upon results of insults to the pregnant uterus itself! (cf. Mulon, 36; also Drips, 6a).

[10] In Sokoloff's (19) castration experiments the circular muscles of the uterus seem to be the first to atrophy.

Fränkel (8) says (pp. 26–27):

> Die Oberfläche der sonst hochrotten Eikammern wird blass gelb, längsstreifig und runzelig, ihre pralle Spannung verliert sich, weil sich als erstes Zeichen der beginnenden Rückbildung die Fruchtwassermenge verringert; die Consistenz der Kammern wird dadurch hart und höckerich, die Form derselben, die vorher der Kugel am nächsten stand, wird mehr länglich, cylindrisch.

The French authors, too, contend that the corpora lutea are necessary for the "serous infiltration" into the connective tissue spaces of the mucosa as well as for the mitotic growth of the cellular elements. The lack of "serous infiltration" into the vast spaces of the opossum endometrium, in which widely separated blood vessels and glands are normally suspended —this is the most striking change in the uteri of my experimental animals. I attribute the malnutrition and death of embryos directly to it (cf. figs 7 and 8).

These phenomena are recorded by Fränkel and by Bouin and Ancel and have been observed by myself. They are passed over with a gesture by Loeb, whose decidual reaction he seems to regard as the sole factor involved.[11]

6. Two ovarian factors operate in stimulating the growth of the uteri. Before ovulation it is the follicular fluid (Robinson, Marshall and Wood Adler, Schröder, Hartman, etc.). After ovulation the corpus luteum is responsible for the progressive changes in the uteri, although a few still consider the ovum solely responsible (Schröder, R. Meyer). Adler takes Fränkel to task for overlooking the first phase in the pre-gravid development of the endometrium, but this is not a serious indictment and should not detract from the worth of Fränkel's larger contribution.[12]

There is, however, one other factor which has heretofore not been considered, namely, the influence of the liquor folliculi which Sobotta (48) has shown is expelled into the oviduct with the eggs at ovulation (mouse, rabbit guinea pig). This fluid is doubtless slowly absorbed and in the light of Allen and Doisey's work (1) must be seriously considered in this connec-

[11] Compare, however, Loeb and Hunter (28) p. 297: "The ability of producing decidua is, therefore, not itself sufficient for the development of pregnancy."

[12] This also holds true with reference to Fränkel's notion of a causal relation between the corpus luteum and menstruation. On this point Evans (7, p. 578) says: "It is true that Fränkel's first formulation of the dependence of menstruation on the corpus luteum in the sense that the former was produced by the latter was erroneous; but the premenstrual endometrium is so produced. He has been sufficiently castigated by many subsequent workers and acknowledged the error of his ways if by no other evidence than by his recent reformulation in the Liebmann Handbuch. This should not cause us to overlook Fränkel's establishment of the connection between the corpus luteum and the uterus both in the cycle and in implantation. The necessary presence of the ovary for menstruation had been demonstrated by the classic transplantation experiments of Halban."

tion. It may well be that the follicular fluid is actually, after ovulation as before, the "nutritive substance" of the ovum postulated by Kohn (21) who would derive it from the corpus luteum.

That the embryo requires a continued supply of ovarian hormone for its development seems doubtful, however. Bird, reptile and monotreme eggs develop without it, unless indeed the egg has this substance stored in the yolk or the albumen. Brachet (4) observed rabbit eggs develop an ectoplacenta in vitro, using blood plasma, both male and female, as the culture medium. In the present experiments and in those of Fränkel and of Biedl, Peters and Hofstätter the ova underwent considerable development before they died. However, in these cases, (including the more sucessful transplants of Biedl, Peters and Hofstätter) the ova were subjected to the possible influence of the liquor folliculi absorbed from the fallopian tube. In any event the fact remains that embryos may arise from fertilized eggs at least without the nursing influence of a corpus luteum. Whether the liquor folliculi is sufficient must be determined by experiment.

It seems certain, then, that the embryos die after ovariectomy of the mother because of uterine inefficiency. In other words, the ovarian hormone acts on the uterus, not directly on the embryos, for the effects of spaying are identical in pseudopregnant and in pregnant females. Indeed, as Fränkel pointed out, normally the uterus undergoes pre-gravid enlargement far out of proportion to the growth rate of the eggs.

7. According to Fränkel, then, the trophic disturbance caused in the uteri by the ablation of the corpora lutea results in a faulty implantation of the embryo. Loeb's results are more definite: in the non-pregnant rabbit or guinea pig there is no decidual reaction in the absence of a young active corpus luteum. By inference the principle holds also in pregnancy and this seems perfectly reasonable. But in view of Biedl, Peters and Hofstätter's results, who report, though with reserve, cases of implantation and decidual formation without a corpus luteum, it would seem desirable that the Loeb theory be established also for normal pregnancy.

It may well be, indeed is quite likely, that the decidual reaction is indispensable in certain mammals, at least in those thus far studied experimentally—a cenogenetic character added in the phylogeny of the series. But the foregoing experiments on the opossum and their agreement with those recorded for the rabbit (Fränkel, Bouin and Ancel) demand that we retrace our steps and see whether or not there are factors of importance involved other than the decidual reaction, a phenomenon which now dominates the field. Certainly the decidual reaction has nothing to do with the opossum which has no placenta, no decidua, nor any rudiment thereof. Yet ablation of the ovaries results in deleterious changes in the uterus that kill the embryos. How does the ovarian hormone cause the uterus to maintain its fulness and turgidity, determine the perfection of the circulation and the

exudation of lymph into the endometrium? The factors underlying these "general" and therefore more elusive phenomena have been ignored by Loeb, just as the decidual reaction has been ignored by Fränkel and by Bouin and Ancel.

That there are other factors also in the rodents, the rabbit for example, is shown by the futher fact that the embryos die if the mother's ovaries are removed even after the ninth day, when the decidual reaction has been completed and implantation has been effected (Niskoubina, Magnus).

8. There are other incidental observations worthy of mention. One of these is the great variability in the effect of ovariectomy on the extent of degeneration found in the uteri. As a general rule the uterus undergoes a perfectly normal if somewhat hastened involution, somewhat like the puerperal organ; and the dead fetuses are resorbed as in normal pregnancy, in which usually one or more dead fetuses are to be found in the overpopulated organ. In figure 7 is seen, for example, a uterus in which nothing pathological can be detected in the cellular elements of the basal portion of the mucosa. But some uteri show greater degeneration, as for example 605 (fig. 9) and other cases are recorded in the protocols. No. 795 constitutes the most extreme case. On superficial examination the uterus seemed not unduly degenerated (fig. 4), but the mucosa was found to be in an advanced stage of necrosis, clouds of polymorphonuclear leucocytes infiltrating the whole mucosa and the atrophic glands (fig. 10).

This variability Fränkel (8) has also recorded for the rabbit uterus, his cases XLIV and XLVI being comparable to the two last mentioned above.

9. There is also a great difference of resistance among the embryos, even of the same litter. The entire contents of the uterus may be uniformly macerated (no. 602, fig. 2) or a single embryo may survive the others (no. 749). The embryos may die at different stages in the same uterus, as in no. 604. In a single case the eggs died about the primitive streak stage (no. 925) and in another the embryos were still alive eight days after the operation. This differential viability is a common phenomenon in nature. It holds for the ovarian eggs, for the embryos of a normal uterus (51), (41), (14) and for the population after birth. As to the explanation of the phenomenon, the factors are too multitudinous for discussion here.[13]

## SUMMARY

1. Thirty double and many unilateral ovariectomies were performed upon opossums at various stages of pregnancy and the oestrous cycle.

2. Unilateral castration is practically without effect on the oestrous cycle or the course of pregnancy. Laparotomies are not greatly prejudicial to the animal.

[13] It is interesting to note that in these cases as well as those of Sokoloff (49) and others there is death and resorption, *not* abortion of the embryos (cf. Koebner, 20, and Drips, 6a).

3. As in the higher mammals, castration snuffs out the cyclic changes in the accessory reproductive organs, which return to the resting condition.

4. Removal of the ovaries in pseudopregnancy results in a more rapid involution of the uterus. Involution usually proceeds in a normal fashion, though occasionally there may be an abnormal amount of degeneration of the endometrium.

5. The ovaries are as necessary for the continuance of pregnancy in the opossum as in the placental mammals thus far studied; for double ovariectomy early in pregnancy *invariably* causes the death of the embryos.

6. The direct cause of death in these cases is malnutrition of the embryos due to the collapse of the central layer of the uterine mucosa from which the lymph is resorbed. The uterus becomes flaccid and pale and assumes a peculiar elongated cylindrical form.

7. The failure of implantation in these experiments cannot be due to an interference with the "decidual reaction" of Loeb, now tacitly considered the *sina qua non* of implantation, for in the opossum there is no placenta, no decidua.

8. Since, furthermore, castration early in pregnancy in the rabbit, the guinea pig, the rat, and the dog has as a consequence a palpable interference with the nutrition of the pregnant uterus, it is suggested that the "decidual reaction" of Loeb, beautiful as this phenomenon is, is not the fundamental factor in the ovarian control of implantation.

9. It appears necessary, therefore, that we retrace our steps and consider anew the trophic theory of Fränkel, vague though this be at present, and seek to discover the mechanism by which the ovary controls the nutrition, circulation, secretion, and the muscular tonus of the pregnant uterus.

10. Since the follicular fluid of the ovary has been shown to cause the pro-oestrous pre-gravid hyperplasia and swelling of the endometrium, it is possible that it may continue its effect while being resorbed from the fallopian tube into which it is ejected with the ova at ovulation.

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## PROTOCOLS OF OVARIECTOMIZED ANIMALS

*Note:* All of the 30 spayed animals as well as the 3 special semi-spayed controls listed below (except 237 to 252) were operated after the first ovulation of the breeding season. Since during anoestrum (middle of November to middle of January) the reproductive organs are in a state of rest (practically infantile or pre-pubertal), the experiments are for the most part uncomplicated by previous oestrous changes in

uterus or mammary glands. In January, therefore, each female, though fully mature, is practically in the condition of a virgin animal, a clear advantage over the continuously breeding laboratory mammals.

*No. 154.* Both ovaries removed December 13, 1915, in anoestrum. Corpus luteum fed until February 9, when palpation of the mamary glands seemed to indicate some sexual activity. At autopsy it was found that a small portion of the ovary had been retained, 4 or 5 large graafian follicles being found. Mammary gland on sectioning was found to be in the condition of early prooestrum: edematous; mitoses in the acini. Uterus as in anoestrum.

*No. 155.* Ovariectomy December 31, 1915. Corpus luteum fed until March 13, the mammary glands remaining thin throughout the experiment. Killed April 13; all the reproductive organs in an infantile condition.

*No. 181.* Young virgin female. Ovaries removed January 20, 1916. Killed February 20; organs in an infantile condition.

*No. 186.* Pregnant female, normal embryos of 8 mm. H. R. length. January 22, 1916, left uterus and both ovaries removed. Killed 4 days later. Abortion had taken place and uterus was greatly degenerated and excessively hemorrhagic, small groups of glands immersed, as it were, in blood. Milk could be pressed out of the greatly hypertrophied mammary glands which were like normal lactating organs.

*No. 237.* February 17, 1916, removed both ovaries and left uterus. Latter contained 6 opaque eggs about 7 or 8 days old. Killed 16 days after operation. All reproductive organs greatly retrogressed as compared with no. 235, a control animal in which but one ovary was removed. In the latter the uterus, though small, remained vascular and the lateral vaginal canals were hypertrophied. Its ovary was compensatorily enlarged (370 mm. $^3$) by virtue of the growing follicles.

*No. 239.* Animal, about 10 days pseudopregnant, ovariectomized February 17, 1916. Killed 20 days later; all reproductive organs including mammary gland had reached the resting anoestrous condition. A control animal (no. 236), semispayed on February 17 and also killed 20 days later, was in mid-prooestrum and had an ovary measuring 394 mm.$^3$. In the latter animal the mammary glands retrogressed much more slowly than in spayed no. 239.

*No. 241.* Spayed in early pseudopregnancy, February 20, 1916. At the same time no. 240, in exactlythe same condition, was semi-spayed. Twelve days later uteri and later vaginal canals of the two animals were of about the same size; those of no. 241 were the more pallid. The mammary glands of the spayed animals were very thin, whereas those of no. 240 (semi-spayed) were still very thick.

*No. 248.* Similar to no. 239, with only a 10-day interval after ovariectomy.

*No. 252.* Ditto. Thirteen day interval, mammary gland was still rather thick, but considerably involuted and vacuolated.

*No. 554.* January 25, 1921, both ovaries and left uterus removed. Thirteen eggs with crescentic ova recovered. These had not been in the uterus over 24 hours. Killed 6 days less 5 hours later. Mammary glands flaccid to the touch but in section showed considerable development. Mitosis had ceased and the acini were thin-walled (cuboidal cells) and dilated. The changes had, therefore, been progressing as in psuedopregnancy, though somewhat abortive. Vagina and lateral vaginal canals as in the normal animal: L and E stage in the former; great desquamation in the latter. Uterus normal with the exception of reduction in size and lack of turgor. Glands and epithelium practically like figure 24, pl. 5, Hartman, 1923.

No. *555.* January 25, 1921, both ovaries and uterus removed; latter contained 5 young embryos. Killed February 22; organs in the resting condition (fig. 6). Mucosa of uterus greatly reduced; mammary glands 1 mm. in thickness, alveoli

few but evidencing former activity by their dilated condition. Many alveoli in process of degeneration.

*No. 573.* January 27, 1921, double ovariectomy. Left uterus contained large embryos (number not recorded). Organ very turgid, two chorionic vessels emerged through a hernia caused by a slit in the musculature (fig. 11). Killed January 29; right uterus, although it contained apparently 12 normal fetuses near term, was somewhat flaccid, elongated and purple. Regressive changes in two days were noticeable but not marked. No degeneration recognizable in section of uterus and adhering chorions. Mammary glands greatly hypertrophied, reaching a thickness of 8 mm. as measured in the alcohol specimen, a maximum dimension for pregnant animals.

*No. 580.* February 1, 1921, double ovariectomy. Left uterus yielded 2 mm. vesicles with embryos in the primitive streak stage (see fig. 43, pl. 9, Hartman, 1921). Killed 4 days, 19 hours later. Result practically the same as illustrated by no. 602, figures 1 and 2. Uterus collapsed, but otherwise apparently normal as seen in section, although embryos were in an advanced stage of necrosis (fig. 7). Mammary gland seems considerably degenerated; acini thin-walled and greatly dilated.

*No. 581.* January 27, 1921, double ovariectomy. Left uterus contained young blastocysts at end of entoderm formation (like those shown in fig. 1, pl. 9, Hartman, 1919). About three days later animal died. Uterus about same as in preceding.

*No. 582.* January 27, 1921, both ovaries removed, also left uterus which contained nine 1-mm. bilaminar blastocysts. Three days less 6 hours later the right uterus contained 8 mm. vesicles practically normal. Uterus was practically normal in appearance and turgidity.

*No. 583.* January 27, 1921, both ovaries were removed with the left uterus, which contained young unfertilized eggs. Animal was killed only two days later, with some retrogression apparent in the uterus; but the mammary glands were recorded as having thickened in the interval.

*No. 585 (control).* January 28, 1921, left uterus and right ovary removed. Former contained 1 mm. blastocysts. Five days less 3 hours later animal was killed. Surviving ovary found amid adhesions; uterus and embryos perfectly normal. Section through uterus and an embryo shown in figure 8.

*No. 594.* January 30, 1921, double ovariectomy. Left uterus contained unfertilized eggs about 2 days old. Nine days later animal was killed. Uterus greatly involuted (fig. 5); involution much hastened in comparison with normal controls. Uterine glands filled with cellular debris and epithelium vacuolated. In section the mammary glands seem to be pretty well filled with acini, many of which are dilated. No mitoses. The organ therefore gives evidence of progressive though abbreviated development as in pseudopregnancy.

*No. 602.* January 30, 1921, both ovaries removed. Eighteen eggs in cleavage (about 16-celled) taken from left uterus (fig. 1a) . Left ovary with 18 corpora lutea weighed 252 mgm.; right with 8 corpora, 135 mgm. Killed 8 days later. Uterus much reduced in size and flaccid as compared with the normal pregnant organ (fig. 1). Embryos with amnion and chorion recognizable but long dead and undergoing disintegration (fig. 2). Uterine glands and epithelium appear quite normal in section. Mammary glands exhibit considerable activity—almost like a lactating gland: acini thin-walled (single layer of cuboidal cells) and much dilated: no mitoses.

*No. 604.* Both ovaries removed January 31, 1921. Left uterus yielded 12 eggs, mostly young blastocysts like that shown in figure 1, plate 8, Hartman, 1919. Killed 6½ days later. Uterus elongate, subnormal in size, with little turgor. Embryos: total number not recorded, but the photograph of the fresh open uterus shows 5 or 6.

One of these was fairly normal but its chorionic vesicle was reduced in size; one embryo was dead, the remainder was reduced and moribund. The latter still showed some division figures in the sections. The uterus, with its glands and its epithelial folds interdigitating with the embryonic chorions, seemed quite normal in section.

*No. 605.* January 31, 1921, removed both ovaries; also left uterus containing 14 unfertilized eggs about a day old. Seven days later 5 much encrusted eggs were removed from the uterus. This organ was much reduced in size, like no. 602, but was much more degenerated. Involution exceeded that of no. 594 (fig. 9).

*No. 616.* February 1, 1921. Both ovaries removed; also left uterus, which contained two large embryos. Killed 2 days later. Surviving uterus, apparently normal, contained six normal embryos and two dead vesicles. The latter had died before the operations. Two days marked practically no effect of ovariectomy.

*No. 743.* January 24, 1922, both ovaries removed together with left uterus. This contained about 10 eggs in early stage of fragmentation. Animal died night of January 27 to 28. Uterus was found to have the characteristic elongated shape following ovariectomy.

*No. 794.* January 20, both ovaries removed together with left uterus. Fifteen eggs in about the 16-celled stage recovered. Eight days later, surviving uterus was found to be subnormal in size (fig. 4) and quite flaccid. Only one embryo survived disintegration (fig. 12). Mammary gland 3.0 mm in diameter.

*No. 795.* Both ovaries removed January 24, 1922. Left uterus contained 8.0 mm. vesicles with embryos of about 10 somites. Killed 5 days later. The small and elliptical uterus did not seem unusually degenerated on superficial examination, but on opening only a cloudy fluid was found within. This proved to be due to a profound leucocytosis which affected the entire mucosa. Only the basal layer of glands remained fairly intact (fig. 10). The corresponding lateral vaginal canal was likewise distended with the pus-like fluid. The mammary gland, 5.6 mm. in thickness, resembled a normal gland at parturition.

*No. 804.* January 21, 1922, both ovaries removed; eggs had just reached the uterus. Killed 8 days later; uterus in anoestrous or resting condition. Involution unusually complete and rapid. Indeed, the uterine glands were practically reconstructed; and most surprising of all, contained some mitotic figures.

*No. 813.* January 21, 1922, both ovaries removed. Left uterus contained large embryos. Animal found dead January 25; uterus, greatly reduced in size, contained embryos near term: 2 practically normal, 4 abnormal.

*No. 829.* January 20, 1922, both ovaries removed, also left uterus; this contained 9 embryos in "head flexure" stage. Killed 4½ days later. Uterus, reduced in size and flaccid, contained 4 hemorrhagic embryos near term. Involution of uterus well under way; mucosa massively infiltrated with leucocytes; glands filled with debris and epithelial lining swollen and vacuolated. Mammary gland had reached a thickness of 7.0 mm.

*No. 841 (control).* January 21, 1922, removed *left* ovary and *right* uterus. Latter contained 15 eggs in cleavage (fig. 3a). Eight days, 15 hours later remaining uterus was found to be in perfect condition as to size and turgidity (fig. 3). It contained 4 large embryos. (See fig. 32, pl. 7 and fig. 26, pl. 6, Hartman, 1923, for sections through this uterus.) Mammary gland 6.3 mm. thick.

*No. 889.* This animal was killed February 23, 1922. The ovaries had been removed in January but no record made of the operation. The reproductive organs were in the anoestrous condition.

*No. 925.* January 30, 1924, both ovaries and left uterus removed; eggs in 16-celled stage recovered. Killed 8 days later. Very small uterus contained two dead vesicles 1 mm. and 2 mm. in diameter, respectively.

*No. 927.* January 31, 1924, both ovaries removed. Eggs in left uterus in stage of small blastocysts like those of no. 604. Killed 6 days later. Uterus small and elongate; turgor absent. Two practically normal embryos (hearts beating) and one dead embryo recovered.

*No. 931 (control).* January 29, 1924, removed right ovary and left uterus. Latter contained eggs a little in advance of those of no. 927 and of no. 604. Killed 6 days later. Uterus of normal size and turgidity. Graphic record made of its spontaneous motility. In order to secure a ring of the musculature, several embryos were inadvertently destroyed. Two normal and two retarded embryos were removed entire.

# THE INFLUENCE OF ALKALIES ON THE SECRETION AND COMPOSITION OF GASTRIC JUICE

## I. The Effect of the Prolonged Administration of Sodium Bicarbonate and Calcium Carbonate

THEODORE E. BOYD

*From the Hull Physiological Laboratory of the University of Chicago*

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Certain clinicians, in personal communications to Dr. A. J. Carlson, have reported a temporary achylia following the administration of alkalies over a period of some weeks. This work was started, at Doctor Carlson's suggestion, to test the possibility of producing a similar effect on experimental animals.

There is a considerable volume of literature dealing with the effects on gastric secretion of single doses of the alkaline salts. The reports are so conflicting, however, that no satisfactory conclusion can be drawn from them. So far as I can learn, practically no experimental work has been done on extended alkali feeding with respect to gastric secretory function. Pavlov (10) gave sodium bicarbonate to dogs with their food for several weeks. In subsequent sham-feeding experiments with the same animals he observed a diminished response. The figures from the experiments are not given in his book. Jaworsky (5) stated that long-continued use of the Carlsbad waters had led to chronic gastritis, with permanent absence of pepsin and of HCl from the stomach. Linoissier and Lemoine (6) reported that the Vichy waters, taken in large amounts over a considerable time, caused a reduction in secretion. They asserted that moderate amounts caused stimulation.

Methods. The difficulty of measuring the secretory activity of the intact stomach, even under normal conditions, is well known. It is greatly increased, of course, when alkalies are being fed from time to time. For this series of experiments it was decided to use dogs with accessory stomach pouches. This method also has obvious limitations. The alkaline salts may have a direct action on the gastric mucosa, either in the way of stimulation or of injury and depression. Until we know definitely the mechanism by which the Pavlov or the Heidenhain pouch responds to food, we cannot say to what extent a local depression or stimulation in the main stomach is reflected in the secretory activity of the pouch. It seems probable,

however, that the pouch gives a fairly accurate picture of conditions in the stomach.

Three of the dogs used were prepared by the Pavlov method. In the fourth, the pouch was completely severed from the stomach. No experiments were carried out until at least a month after the operation, and all the animals used were in excellent condition. The arrangement for collecting gastric juice was as described by previous workers in this laboratory (3).

The first experiments were run with two Pavlov pouch dogs, sodium bicarbonate alone being administered. One hour's secretion was collected each day from 12 m. to 1 p.m., before feeding. At 1 p.m. the animals received 200 cc. of water and the stock laboratory meal of meat and table scraps. A fixed standard meal might at first thought seem preferable in order to obtain comparable results. It is a matter of common observation, however, that the dog's appetite is almost as susceptible to monotony as is that of the human. This would probably cause a depression of the appetite secretion after a month or so of the same daily diet. The sodium bicarbonate feeding was carried on for more than two months, with ten or twelve days' control before and after. This period seems sufficiently long for the average figures to give a reliable indication of the effect of the alkali.

The gastric juice was collected for a period of $2\frac{1}{2}$ hours after feeding. Water was then kept in the cages until 8 or 9 p.m., after which it was removed and no more given until the following day's experiment.

Sodium bicarbonate was given in perforated capsules. These can be held between the tips of two fingers and placed on the back of the animal's tongue, when they are readily swallowed. At first the alkali was given only during the digestion period, beginning 15 minutes after feeding and at intervals of 45 minutes thereafter. Soon other doses were added, distributed through the day, but about half the total daily dose was given during the digestion period (see protocols). The gastric juice was titrated for free and total acidity by means of dimethylaminoazobenzene and phenolphthalein.

The attempt was made in this way to give sodium bicarbonate with no other drug to complicate the results. Bones were given in plenty with each feeding. In one animal, however, a persistent diarrhea developed, growing worse as the dosage of alkali was increased. The other dog showed diarrhea only at times, but vomited frequently after the alkali was given, both before and after feeding. These symptomsdis appeared within two days after the alkali was dropped.

Experiments were then carried out on two other dogs, one with a Pavlov and one with a Heidenhain pouch. The procedure was modified in several respects. Sodium bicarbonate was mixed with an equal quantity of cal-

cium carbonate, in an attempt to prevent the appearance of the diarrhea produced in the earlier experiments. The total daily dose of the mixture was divided into four equal parts, and given by stomach tube. The first was stirred into a constant quantity of milk (dog 3, 250 cc., dog 4, 400 cc.) and given at 9:00 a.m. The three remaining doses were given, each in 150 cc. of water, at 1:15, 5:00 and 9:00 p.m., respectively. The dogs each received the stock laboratory meal at 1:00 p.m. Separate records and titrations were made of *a*, one hour's continuous secretion, from 8:00 to 9:00 a.m.; and *b*, four hours' secretion after the milk was given (9:00 a.m. to 1:00 p.m.).

Besides the quantity and acidity of the gastric juice, the following data were recorded:

*1. Peptic activity of the gastric juice.* This was not determined every day. Several samples were tested during the control periods and during the alkali administration, to see if any marked change was produced. Mett's tubes were used, the gastric juice being diluted with 15 volumes of 0.3 per cent HCl and kept at 37°C. for 24 hours.

*2. Free acid in the main stomach.* A sample of the stomach contents was aspirated each day just before feeding at 1:00 p.m., and tested for free acid, with dimethylaminoazobenzene. It was not titrated.

*3. Reaction of the urine.* The urine was tested with litmus each morning, before any alkali was given.

*4. Amount of water intake each day.* Large doses of sodium bicarbonate are followed by evidence of marked thirst, as in the case of any other soluble salt. The available water content of the body is of considerable importance with respect to gastric secretion, as shown by the work of Sutherland (13) and others. The discomfort of thirst may also cause some degree of inhibition.

*5. The chloride content of the plasma.* This was measured at intervals of three days. The blood was drawn early in the morning, before alkali or water was given, and the chlorides determined as NaCl by the method of Van Slyke and Donleavy (14).

*6. The carbon dioxide capacity of the whole blood.* This was determined at the same time as the preceding, at intervals of three days. The Van Slyke method was used. N/10 lactic acid was employed instead of sulphuric, according to the modification described by Van Slyke and Stadie for use with whole blood (15). No corrections were made for temperature or barometric pressure.

7. The volume ratio of corpuscles to whole blood was noted on each sample of blood drawn for the above tests, as an aid in interpreting any changes in the chloride content or $CO_2$ capacity.

RESULTS. *1. On feeding sodium bicarbonate alone.* The mean results are shown in tables 1 and 2. In both cases, the secretion rate was higher

during the administration of the alkali than during the first control period. The daily record was too long for inclusion. As stated above, it shows

TABLE 1

*Dog 1. Female. Operated on January 4. The alkali was given from April 2 to June 14. Weight, April 1, 8.6 kgm., June 14 8.8 kgm. The animal was fed the stock laboratory meal at 1:00 p.m., daily, and the gastric juice was collected for 2½ hours afterward*

| DAYS | TOTAL $NaHCO_3$ DAILY | SCHEDULE | GASTRIC JUICE (MEAN) | FREE ACID (MEAN) | REMARKS |
|---|---|---|---|---|---|
| | *grams* | | *cc.* | *per cent* HCl | |
| 10 | | Control | 7.9 | 0.31 | |
| 20 | 2.7 | 0.9 gram at 1:15, 2:00 and 2:45 p.m. | 7.8 | 0.25 | |
| 23 | 4.5 | Added 0.9 gram at 11:00 a.m. and 5:00 p.m. | 9.2 | 0.31 | Vomited after eating on 5 days |
| 17 | 6.3 | Added 0.9 gram at 8:00 a.m. and 9:00 p.m. | 11.7 | 0.33 | Vomited after eating on 4 days. Refused to eat 2 days |
| 15 | 12.6 | Doubled all doses | 12.4 | 0.33 | Vomited after eating on 4 days |
| 12 | | Control | 13 3 | 0.30 | |

TABLE 2

*Dog 2. Old female. Operated on February 3. Record begun March 23. Ended June 14. Alkali administered from April 5 to June 2. Weight, April 4, 20.2 kgm. June 2, 19.7 kgm. The animal was fed the stock laboratory meal at 1 p.m. daily. Gastric juice collected for 2½ hours after feeding*

| DAYS | SCHEDULE | TOTAL $NaHCO_3$ | GASTRIC JUICE (MEAN) | FREE ACID (MEAN) | REMARKS |
|---|---|---|---|---|---|
| | | *grams* | *cc.* | *per cent* HCl | |
| 12 | Control | | 13.0 | 0.24 | |
| 20 | 0.9 gram at 1:15, 2:00 and 2:45 p.m. | 2.7 | 12.8 | 0.27 | |
| 8 | Added 0.9 gram at 11:00 a.m. and 5:00 p.m. | 4.5 | 13.8 | 0.24 | Diarrhea on 2 days |
| 14 | Added 0.9 gram at 8:00 a.m. and 9:00 p.m. | 6.3 | 14.5 | 0.26 | Diarrhea on 4 days |
| 17 | Doubled doses at 1:15, 2:00 and 2:45 p.m. | 9.0 | 20.7 | 0.29 | Diarrhea on 7 days |
| 12 | Control | | 24.4 | 0.32 | |

frequent gastro-intestinal disturbances with the larger doses. The lowest secretion rates observed occurred on the days when the diarrhea or vomiting was most severe.

A feature of both records is that the rate of secretion during the second control period was highest of all. The daily records show that a high level was maintained for six or seven days after the alkali was dropped. The rate then gradually fell, approaching the level of the first control.

After the alkali feeding was discontinued, the diarrhea and vomiting stopped. The secretion rate was more uniform thereafter, which accounts, in part at least, for the higher average during this period. There were individual days, when the alkali was being given, on which the secretion rose to a very high level, but the days of gastro-intestinal upset reduce the averages.

TABLE 3

*Dog 3. Female, weight 9.7 kgm. Pavlov pouch made June 19, 1922. This experiment started June 3, 1923*

| DAYS | | AVERAGE AMOUNT | FREE ACID |
|---|---|---|---|
| | | *cc.* | |
| 5 | Control | 12.4 | 0 15 |
| 7 | −10 grams each of $NaHCO_3$ and $CaCO_3$ daily | 16.8 | 0.21 |
| 14 | 15 grams of each daily | 14.7 | 0.15 |
| 5 | 30 grams of each daily | 8.3 | 0.10 |
| 4 | 60 grams of each daily | 4.4 | 0.04 |
| 8 | Control | 19.6 | 0.29 |

TABLE 4

*Dog 4. Female, weight 19.6 kgm. Heidenhain pouch, made January 4, 1923. This experiment was started May 29, 1923*

| DAYS | PROCEDURE | GASTRIC JUICE COLLECTED FOR FOUR HOURS AFTER FEEDING (AVERAGES) | |
|---|---|---|---|
| | | Amount | Free acid |
| | | *cc.* | |
| 7 | Control | 11.9 | 0.16 |
| 7 | 20 grams each of $NaHCO_3$ and $CaCO_3$ daily | 12.8 | 0.14 |
| 16 | 30 grams of each daily | 10.7 | 0.09 |
| 7 | 60 grams of each daily | 9.2 | 0.09 |
| 9 | Control | 15.0 | 0.23 |

In these animals, then, no diminution of secretion was ever produced without being accompanied by signs of severe irritation of the gastro-intestinal tract. In the absence of these symptoms, the secretion was usually higher than normal.

*2. Feeding sodium bicarbonate with calcium carbonate.* The average for these experiments are given in tables 3 and 4. Symptoms of gastro-intestinal irritation were not so much in evidence in these cases. Both animals vomited a few times while the larger doses were being given, and

dog 3 showed diarrhea on three days. The vomiting, when it occurred, nearly always followed the last dose of alkali in the evening. This dose was given on the empty stomach. When given in milk, or following the 1:00 p.m. meal, it was better tolerated. Toward the close of the alkali period both dogs lost appetite and appeared rather listless and inactive.

Both animals showed a pronounced depression of secretion when the daily dosage was raised to 3 grams each of sodium bicarbonate and calcium carbonate per kilo body weight per day. With half that amount there were days when the secretion was quite low, but they were balanced by an unusually high rate on other days. The secretion rate varies greatly even under normal conditions, and this irregularity becomes much more marked under alkali feeding.

A rise in secretion after the alkalies were stopped was quite distinct with all the Pavlov pouch dogs. The one Heidenhain pouch animal showed a similar rise, but of less degree. Whether this represents a real difference of reaction between the Heidenhain and the Pavlov pouch is uncertain, since only one of the former type was used. As to the mechanism of the hypersecretion, I have at present no theory to offer.

*Pepsin concentration.* There were the usual variations in peptic activity, but not beyond the normal range. There is nothing in the results to indicate that the concentration of pepsin is affected in either direction by the alkali given.

*Acidity in the stomach.* The routine testing of the stomach contents, four hours after feeding the milk, showed results as follows:

Dog 4 showed free acid present on eleven out of sixteen control days, and on eight out of thirty days while alkali was being given. Dog 3 showed free acid present on ten out of thirteen control days, and nine out of thirty days of alkali feeding. These results merely show that no serious injury was done to the gastric glands directly. Free acid was present as often during the second control period as during the first. The infrequent appearance of free acid in the stomach during the alkali feeding was doubtless due largely to direct neutralization by the alkali.

*The reaction of the urine.* Dog 4 showed alkaline urine throughout the entire period of alkali feeding. The urine of dog 3 was twice acid to litmus, but remained alkaline after the dosage was raised to 1.5 grams each of $NaHCO_3$ and $CaCO_3$ per kilo body weight.

The water intake varied, but increased with alkali feeding, roughly in proportion to the dosage. The average amounts of water taken per day during the control periods were, for dog 3, 280 cc.; dog 4, 430 cc. During the period of maximum alkali dosage the average intake of dog 3 was 800 cc.; of dog 4, 1300 cc.

*The chloride content of the plasma.* Figure 1 shows the results. It has been observed by Haldane and co-workers (1), (2) that when a high blood

chloride content is produced by giving $NH_4Cl$ or $CaCl_2$, the bicarbonate content is diminished, the sum of their molecular concentrations remaining approximately constant. It would seem likely, then, that the administration of bicarbonate might lead to a decrease in blood chlorides. This was the case in my experiments. It should be remembered that the blood used in these determinations was drawn at least ten hours after the last dose of alkali, at a time when recovery from its effects was presumably at a maximum for the day.

*The alkali reserve.* It is probable that this was abnormally high during the greater part of the day. Since the $CO_2$ capacity determinations were

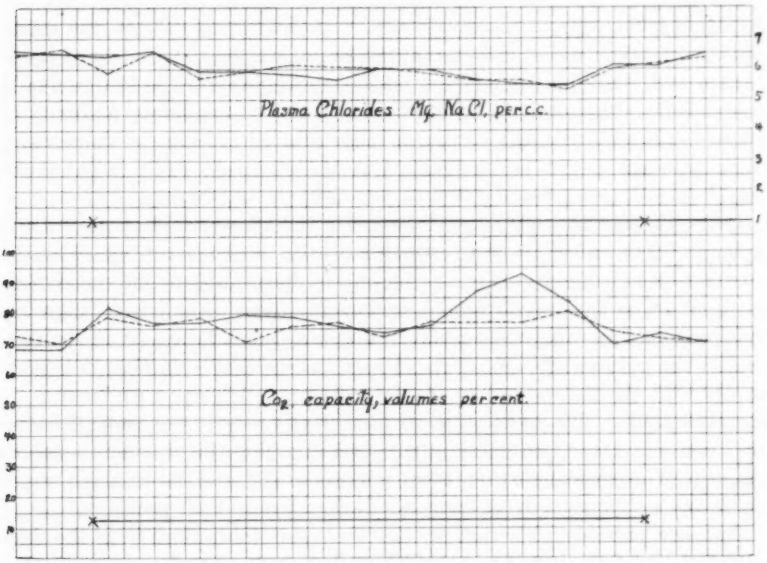


Fig. 1. Chart showing variations in plasma chlorides and $CO_2$ capacity of whole blood. Each interval on the abscissa equals one day. *X*——*X*, period of alkali feeding. The curves for dog 3 are in broken lines, those for dog 4 in solid black.

made early each morning, before alkali was given, they presumably indicate the minimum alkali reserve present at any time of the day. It will be seen from the accompanying chart that this minimum value was as a rule within the normal range, and only slightly above the average normal value for whole blood. Dog 4 shows a more sustained rise than dog 3, although the dosage was carried to a higher amount with the latter animal.

No symptoms of tetany were observed during these experiments.

Discussion. The results here presented assuredly show no after-effect of gastric secretory depression from feeding alkalies. They show exactly

the opposite, that is, hypersecretion. Not even as an immediate effect was any depression observed, until the dosage of the alkalies was carried considerably above that ordinarily used in human therapy. There was in every case a higher rate of secretion with moderate alkali dosage than during the first control period. The figures thus seem to indicate a stimulant effect. One must hesitate at such a conclusion, however, in view of the great spontaneous variations in secretion. It does seem to be a justifiable conclusion that the smaller doses used produce no depression.

What is the mechanism of the depression produced by larger amounts of alkali? This question cannot at present be answered.

Lonnquist (7) presents evidence to show that alkalies inhibit gastric secretion reflexly, from the duodenum. In the human subject, the introduction of alkali directly into the duodenum has been found to produce nausea and vomiting (4). Under these conditions gastric secretion would probably be depressed. The indifferent action of moderate amounts of alkali fits in well with the theory of reflex inhibition from the duodenum. Such amounts might be neutralized in the stomach, while larger doses might furnish an excess of alkali to pass into the duodenum.

One objection to such an explanation is the alleged acid control of the pylorus. According to this conception, the presence of alkali in the stomach would merely delay the emptying time until it had been neutralized. The free alkali would not reach the duodenum. There are evidently factors other than acidity, however, which take part in the control of the pylorus (8), (9), (12). I have carried out a number of experiments with the fluoroscope on dogs, feeding starch paste with barium by stomach tube. The addition of sodium bicarbonate in concentrations up to 7 per cent caused no delay in the time at which the stomach began to empty. The earliest portions of such material to leave the stomach would obviously be more likely to carry alkali than would later portions.

Another possible objection to the theory of inhibition from the duodenum is the fact that the heavy alkali dosage depressed secretion in the Heidenhain pouch as well as in the Pavlov pouch. It is true that there is a greater interference with nerve paths in the former preparation than in the Pavlov type. It is far from being "isolated," however. Recent observations by Robins and Boyd (11) show that inhibitory reflexes may reach the musculature of the Heidenhain pouch from stimuli in the main stomach. Assuming that all vagal connections to the pouch are cut, its secretory activity may still be affected by vasomotor reflexes.

It hardly seems necessary, however, to assume any specific reflex to explain the depression of gastric secretion produced by a dose of 1 gram or more of sodium bicarbonate per kilo body weight. The effect is more likely a complex one, depending on several abnormal conditions. In the first place, alkalosis is produced, probably to a degree sufficient to cause

some physical discomfort. If water is withheld, there is severe thirst. Similar doses cause nausea in human beings. These factors would all tend to a psychic inhibition of gastric secretion, assuming that they have no more direct action. Diminution of chlorides, and concentration of the blood, probably contribute to the result. It is not possible at present to say how far the rise in alkali reserve would interfere chemically with the formation of acid gastric juice, if it does so at all.

## CONCLUSIONS

1. In dogs, the feeding of sodium bicarbonate, in amounts up to 1 gram per kilo body weight per day, produces no diminution in the average quantity or acidity of gastric juice. The rate of secretion becomes more irregular, and the occasional depression which occurs is usually accompanied by symptoms of gastro-intestinal irritation.

2. When the diarrhea is controlled by combining sodium bicarbonate and calcium carbonate in equal amounts, the secretion is not appreciably depressed until the dosage exceeds 3 grams of the mixture per kilo body weight per day.

3. There is evidence that such a dosage of alkali produces a considerable diminution in the blood chloride content. This may account, in some degree, for the depressing effect of large doses on gastric secretion.

4. The depression produced by feeding alkalies in large doses does not outlast the period of administration. Hypersecretion, lasting for several days, usually follows the discontinuance of the alkali.

I wish to thank Dr. A. J. Carlson for many valuable suggestions and criticisms in the course of the work which is reported in this and in the accompanying paper. My thanks are also due to Mr. Stanley E. Lawton for assistance in the operations and experiments.

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# THE INFLUENCE OF ALKALIES ON THE SECRETION AND COMPOSITION OF GASTRIC JUICE

## II. The Effects of Single Doses of Sodium Bicarbonate and Calcium Carbonate

THEODORE E. BOYD

*From the Hull Physiological Laboratory of the University of Chicago*

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In another paper (5) I have reported a series of experiments with alkali feeding over extended periods. The results obtained made it seem desirable to re-investigate the effects of single doses of the alkalies used. The various reports of work in this field are by no means in agreement. The greater number are inclined to ascribe to $NaHCO_3$ an inhibitory effect on gastric secretion. $CaCO_3$ is by some observers classed as stimulant, by others as indifferent or as inhibitory.

The literature. The earliest experimental work on this problem was done on animals with simple gastrostomies. Blondlot (4) reported a long-continued flow of gastric juice, with high acidity, after feeding gastrostomy dogs on meat moistened with sodium carbonate. Claude Bernard (1) mentions similar observations, though I have found no published data of his on the subject. Gilbert (9) reported stimulation of secretion by Vichy water and by sodium bicarbonate solution.

Pavlov (16) used animals with accessory stomach pouches prepared by his method. He found that solutions of sodium bicarbonate of 0.5 per cent to 1 per cent strength, introduced into the stomach in quantities of 150 cc., failed to stimulate secretion as did equal amounts of distilled water. He states, however, that this amount of water alone caused stimulation in only about one-half of the experiments. The evidence for inhibitory action does not therefore seem to be conclusive.

Several later investigators used the Pavlov pouch. Bickel (2) produced a copious secretion from the pouch by the injection of pilocarpine. He reported that the secretion thus produced could be completely suppressed by sodium bicarbonate, after which a second injection of pilocarpine had no effect. This would seem rather strong evidence of a depressant action of the alkali, especially since Ivy (4) has shown that the gastric glands normally will continue for long periods to respond to successive injections of secretagogues. Bickel also confirmed the observations of Pavlov, mentioned above.

Heinsheimer (11) reported inhibition by $Na_2CO_3$, $NaHCO_3$ and bismuth subnitrate. Magnesium oxide had little effect of any kind, while calcium carbonate was strongly stimulant. Lönnquist (14) gave solutions of sodium bicarbonate (0.25 to 1.5 per cent) in amounts of 200 cc., and reported inhibition as a usual result. If the stomach were actively secreting already, however, the alkali caused a further stimulation. Mayeda (15) found that calcium carbonate and lithium carbonate were stimulants. Chiari (6) stated that sodium bicarbonate, sodium carbonate, magnesium oxide and lime water were all inhibitory.

Ehrmann (8) working on animals with denervated stomach pouches, found that sodium bicarbonate (100 cc. of 10 per cent solution) caused a slight increase, both in quantity and acidity, over the normal response to an equal quantity of water. He concludes, therefore, that the inhibitory effect found by previous workers was due to a reflex.

King and Hanford (13) report that 1 per cent $NaHCO_3$, given with food to Pavlov pouch dogs, causes an increased secretion. When given with the stomach empty, it produced less response than equal quantities of water.

There have been numerous clinical studies of the problem, but the results are equally inconclusive. A review is given by Crohn (7).

Such discordant reports are not easy to explain. The gastric secretory response to identical meals varies from time to time within wide limits, and nearly all the figures given are well within the normal range. In view of this, it would seem necessary to obtain the averages from a large number of experiments before drawing conclusions. Most of the reports cited are open to this objection, while others do not state the number of experiments made. If the effect of the alkalies on a Pavlov pouch is really different from that produced on a denervated pouch, variations in surgical technique may be responsible for some of the discrepancies. The experiments carried out in the present investigation are, I believe, sufficient in number to offer some basis for conclusions.

METHODS AND RESULTS. Four dogs were used, one with a Heidenhain pouch and three with Pavlov pouches. The presence of secretory nerves was tested in the latter animals by experiments on "appetite" secretion. None of them gave an invariable response to the sight or smell of food. There was a definite response in about one-half the experiments tried. When the dogs were fed after a fast of 24 to 48 hours, however, the increased flow of gastric juice nearly always began in 5 to 15 minutes, which is earlier than the beginning of the "digestion" secretion, and a shorter time than the latent period of the Heidenhain pouch. All the animals were in excellent condition. No experiment was carried out until they had been at least 18 hours without food and 12 hours without water. In a few cases, the alkali feeding and control experiments were

carried out on alternate days. As a rule, however, intervals of two or three days were given for controls.

The apparatus used for collecting the gastric juice was as described by Hardt (10).

*1. $NaHCO_3$ given after feeding.* Each animal was kept on a constant diet. The food was given at 1:00 p.m. daily. The gastric juice was collected for 1 hour previous to this feeding, and for $2\frac{1}{2}$ hours afterward. With one animal, the total amount of $NaHCO_3$ given at each experiment was divided into three equal doses, which were given in perforated capsules at 15 minutes, one hour and $1\frac{3}{4}$ hours, respectively, after the meal.

TABLE 1

*Dog 2. Old female, weight 20.7 kgm. The daily meal consisted of one pint of milk and two raw eggs. At the end of the experiment each day the animal received 50 cc. of cod liver oil. This particular diet was used because other studies being carried out on the gastric juice at the same time. The total amount of $NaHCO_3$ at each period was given in three equal doses at intervals of 45 minutes, beginning 15 minutes after feeding*

| NUMBER AND TYPE OF EXPERIMENTS | CONTINUOUS SECRETION (1 HOUR) | | SECRETION AFTER FEEDING (2½ HOURS) | |
|---|---|---|---|---|
| | Amount | Free acid | Amount | Free acid |
| | *cc.* | *per cent* HCl | *cc.* | *per cent* HCl |
| 32 control experiments: | | | | |
| Mean results | 1.3 | 0.02 | 13.5 | 0.31 |
| Lowest results of series | 0.2 | 0.00 | 7.0 | 0.23 |
| Highest results of series | 3.2 | 0.17 | 34.0 | 0.46 |
| 6 experiments, 13.5 grams $NaHCO_3$ after feeding: | | | | |
| Mean results | 1.0 | 0.01 | 20.1 | 0.37 |
| Lowest of series | 0.3 | 0.00 | 8.0 | 0.26 |
| Highest of series | 1.5 | 0.10 | 35.0 | 0.51 |
| 2 experiments, 5 grams $NaHCO_3$ after feeding: | | | | |
| Mean results | 1.8 | 0.00 | 22.0 | 0.34 |
| 2 experiments 24 grams $NaHCO_3$ after feeding: | | | | |
| Mean results | 1.3 | 0.00 | 2.7 | 0.02 |

The object was to keep some of the alkali present in the stomach throughout at least the greater portion of the experimental period. Table 1 shows the results.

It will be seen that when 24 grams of $NaHCO_3$ were given in this manner to the 20 kgm. dog, there was a sharp and unmistakable depression of secretion. With the smaller doses the change, when any followed, was usually in the direction of an increase. It may seem probable that the large dose required to bring about any depression was due to the method of administration. Although the capsules were freely perforated,

it may be that only a comparatively small part of the alkali was actually free in the stomach at any one time.

With two other Pavlov dogs, however, experiments were run in which the total amount of $NaHCO_3$ was given by stomach tube 15 minutes after feeding. These animals (dogs 1 and 3) showed results very much alike. Table 2 gives the mean figures for one of them. It will be seen that nearly 2 grams per kilo body weight were required to depress the flow down to the rate of the "continuous" secretion. With the smaller doses, there is evidence of stimulation, as shown by dog 2.

After the large doses of $NaHCO_3$ the animals were as a rule quite thirsty, and drank greedily when water was offered to them at the con-

TABLE 2

*Dog. 1. Female, weight 8.6 kgm. The daily meal consisted of one pint of milk and 200 grams of bread. The total dose of $NaHCO_3$ was given by stomach tube in 100 cc. of water, 15 minutes after feeding. On the control days the water was given alone*

| NUMBER AND TYPE OF EXPERIMENTS | CONTINUOUS SECRETION (1 HOUR) | | SECRETION AFTER FEEDING (2½ HOURS) | |
|---|---|---|---|---|
| | Amount | Free acid | Amount | Free acid |
| | *cc.* | *per cent* HCl | *cc.* | *per cent* HCl |
| 10 controls: | | | | |
| Mean results | 1.9 | 0.03 | 10.0 | 0.26 |
| Lowest of series | 0.4 | 0.00 | 8.1 | 0.18 |
| Highest of series | 3.9 | 0.18 | 13.9 | 0.36 |
| 3 experiments, meal followed by 5 grams $NaHCO_3$: | | | | |
| Mean results | 0.5 | 0.00 | 15.3 | 0.34 |
| Lowest of series | 0.2 | 0.00 | 12.0 | 0.29 |
| Highest of series | 0.9 | 0.00 | 17.2 | 0.38 |
| 1 experiment, meal followed by 10 grams $NaHCO_3$ | 1.9 | 0.00 | 5.6 | 0.11 |
| 1 experiment, meal followed by 15 grams $NaHCO_3$ | 0.6 | 0.00 | 4.1 | 0.00 |

clusion of the experiment. It seemed likely that the depression of secretion observed with these large doses might be due at least in part to the withholding of water. The effect might be caused by a psychic inhibition, from the discomfort of thirst. Or else, the ingestion of any soluble salt in such amounts might tend to raise the osmotic pressure of the blood and so render water less available to the gastric glands.

A series of experiments was carried out to test the importance of the water factor. The animals were fed a standard meal at 8 a.m., each day, and received no other food. One was fed milk and bread (table 3), the alkali being given in perforated capsules and in divided doses, as previously described. The other two were given milk by stomach tube, and the alkali was given in a single dose stirred into the milk. Water

was allowed ad lib. from 8 a.m. to 5 p.m., the entire amount drunk by the animal being measured each day. The entire amount of gastric juice secreted by the pouch during the 24 hours after each feeding was collected and titrated. Collection of a 24-hour sample is practicable with the apparatus used in these experiments, the main source of trouble being the possibility that the bottle may overflow during the night. Since the animals had no food after 8 a.m., and no water after 5 p.m., the secretion rate was as a rule low after 9 or 10 p.m., at which time the bottles were emptied for the last time until early the following morning.

The results obtained with one animal are summarized in table 3. The water intake was considerably increased by large doses of sodium bicar-

TABLE 3

*Dog 3. Female, weight 9.7 kgm. The daily meal consisted of one pint of milk and 100 grams of bread. $NaHCO_3$ was given in three doses after feeding, as described in table 1*

| NUMBER AND TYPE OF EXPERIMENTS | WATER INTAKE | TOTAL GASTRIC JUICE (24 HOURS) | FREE ACID |
|---|---|---|---|
| | *cc.* | *cc.* | *per cent* HCl |
| 23 controls: | | | |
| Mean results | 33 | 97 | 0.34 |
| Lowest of series | 0 | 66 | 0.20 |
| Highest of series | 150 | 184 | 0.41 |
| 3 experiments, with 5 grams $NaHCO_3$ after feeding: | | | |
| Mean results | 183 | 112 | 0.38 |
| 1 experiment, meal followed by 10 grams $NaHCO_3$ | 335 | 142 | 0.40 |
| 3 experiments, meal followed by 15 grams $NaHCO_3$: | | | |
| Mean results | 425 | 143 | 0.35 |
| 2 experiments, meal followed by 20 grams $NaHCO_3$: | | | |
| Mean results | 655 | 35 | 0.09 |

bonate. The depressing effect of such doses was not overcome by allowing unlimited water. With the smaller doses the secretion was greater in amount than in the control experiments. A part of this effect on secretion is doubtless due to stimulation by the water. It was noted, however, that small doses were sometimes accompanied by a marked increase in the flow of gastric juice, without causing any appreciable augmentation in the water drinking. These instances, coupled with the data in table 1, make it apparent that the alkali has of itself some kind of stimulating action, so long as it is not given in excess.

*2. $NaHCO_3$ given on the empty stomach.* In these experiments $NaHCO_3$ solutions, in equal volumes but varying concentrations, were given by

stomach tube. Controls were run using an equal quantity of water. The gastric juice was collected for one hour before this procedure and for three hours after.

No clear evidence of stimulation, by any of the concentrations used, was seen in these experiments. On the other hand, there was on the whole no evidence of inhibition or depression from a 1 per cent solution, as reported by Pavlov, Bickel and others. Solutions of this concentration appear to be indifferent in effect. With the 2 per cent and 2.5 per cent solutions, however, there was a distinct depression. The amount of alkali contained in these doses (dog 3, 0.75 gram per kilo body weight; dog 4, 0.4 gram per kilo) did not produce inhibition when given after

TABLE 4

*Dog 3. Female, weight 9.7 kgm. The daily meal consisted of 200 grams raw hamburger and 100 grams bread. The total dose of $CaCO_3$ was given by stomach tube in 200 cc. of water, 15 minutes after feeding. On the control days the water was given alone*

| NUMBER AND TYPE OF EXPERIMENTS | CONTINUOUS SECRETION (1 HOUR) | | SECRETION AFTER FEEDING (2½ HOURS) | |
|---|---|---|---|---|
| | Amount | Free acid | Amount | Free acid |
| | cc. | per cent HCl | cc. | per cent HCl |
| 37 controls: | | | | |
| Mean of results | 0.8 | 0.00 | 8.7 | 0.26 |
| Lowest of series | 0.0 | 0.00 | 5.0 | 0.05 |
| Highest of series | 1.9 | 0.07 | 12.5 | 0.46 |
| 6 experiments, 7.5 grams $CaCO_3$: | | | | |
| Mean of results | 1.6 | 0.08 | 15.4 | 0.42 |
| Lowest of series | 0.5 | 0.00 | 8.2 | 0.36 |
| Highest of series | 5.5 | 0.47 | 26.5 | 0.49 |
| 5 experiments, 13.5 grams $CaCO_3$: | | | | |
| Mean of results | 1.0 | 0.00 | 12.5 | 0.34 |
| Lowest of series | 0.8 | 0.00 | 5.0 | 0.21 |
| Highest of series | 1.5 | 0.00 | 19.6 | 0.42 |
| 3 experiments, 24 grams $CaCO_3$: | | | | |
| Mean of results | 1.9 | 0.06 | 15.8 | 0.36 |

feeding. The amount required to produce inhibition appears to be less for the empty than for the digesting stomach.

*3. The effect of calcium carbonate given after feeding.* These experiments were similar to those with $NaHCO_3$. Table 4 gives the results with one animal. Calcium carbonate appears to have as a rule a distinctly stimulating effect, in amounts up to 2.5 grams per kilo body weight. Larger doses were not given. This is in accord with the observations of Heinsheimer, Mayeda and others.

*4. The effect of calcium carbonate given on the empty stomach.* The calcium carbonate was given in water suspension, and controls run with an equal quantity of water. The results showed on the average a stimu-

lating effect, though it was somewhat less constant than that observed after feeding.

In all, more than fifty experiments were carried out with $NaHCO_3$ given after feeding, and approximately as many in which it was given on the fasting stomach. The total number of experiments with $CaCO_3$ was about the same, not including controls in either case. The detailed records are too long for publication.

DISCUSSION. In these experiments, as well as in those reported in the accompanying paper, there is some evidence of a dual action of sodium bicarbonate on the secretory mechanism. The first, a stimulation, appears with the smaller doses when they are given following food. It has not been observed following administration of $NaHCO_3$ on the empty stomach. It may be, as suggested by Lönnquist, that this is not a direct effect of the alkali, but an effect of the NaCl or $CO_2$ produced by the intereaction of $NaHCO_3$ with HCl in the stomach. The effect of NaCl on secretion, if it has any, has not been definitely settled. $CO_2$ has been classed as a stimulant (3). The mechanism of its action is not known.

The second effect of $NaHCO_3$, namely, depression from large doses, is much more constant. The mechanism of this effect is also uncertain. Some of the possibilities are discussed in the accompanying paper. A brief note may be added here on the effects observed when $NaHCO_3$ solutions are given on the empty stomach. A relatively small dose given in this manner produced depression. Concentrations up to 1 per cent, however, are not effective.

These observations are perhaps most easily explained on the basis of a reflex inhibition from the duodenum. Water solutions of alkalies, given on the empty stomach, probably are neutralized to a much less extent than when they are given on the digesting stomach. This would be expected for two reasons, first, because the empty stomach contains comparatively little acid; second, because the solutions would probably pass more quickly into the duodenum. The indifferent effect of a 1 per cent solution may be due to the fact that its alkalinity is not far different from that of the normal contents of the duodenum. Doubtless such a solution in most cases is almost if not completely neutralized in passing through the stomach.

*The stimulating action of $CaCO_3$.* It is difficult to see how this substance in its original form could act as a stimulant. The actual stimulation is no doubt produced by $CO_2$ or by $CaCl_2$, formed in reaction with the acid already present in the stomach. If this is so, the stimulation should be more marked if the stomach is actively secreting at the time the $CaCO_3$ is introduced. My observations give no clear indication on this point. Stimulation was frequently observed at times when the continuous secretion of the pouch was almost nil. The $CaCO_3$, however,

was put into the main stomach, not into the pouch. So far as we know the continuous secretion of the pouch may show variations entirely independent of those in the main stomach.

## CONCLUSIONS

1. Sodium bicarbonate may be given to dogs after feeding, in amounts up to about 1 gram per kilo body weight, with no evidence of depression of the gastric secretory mechanism. With small doses the secretion is on the average above normal. Larger amounts reduce the secretion, both in quantity and acidity.

2. When sodium bicarbonate is given with water alone, the dose required to depress secretion is smaller than when it is given after feeding.

3. Calcium carbonate usually produces an increased flow of gastric juice, whether given on the empty or the digesting stomach.

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# THE USE OF DEPANCREATIZED DOGS AS TEST OBJECTS FOR INSULIN

FRANK N. ALLAN

*From the Department of Physiology, University of Toronto*

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With the introduction of insulin into clinical use in the treatment of diabetes there arose the necessity of finding some means of measuring the activity of the extract, not only to permit standardization of different preparations to a uniform strength, but also to serve as a guide in determining the dosage to be given to a patient. As the chief effect of insulin is the restoration of the ability to utilize carbohydrate in the diabetic, the simplest and most logical procedure appeared to be to measure the quantity of insulin in terms of the amount of sugar which can be utilized in the body under its influence.

While this relationship was being studied in the clinic, another means of testing the activity of insulin, through its effect in lowering the blood sugar of normal rabbits, was discovered and developed in the laboratory (1). This has now come to be the most useful and convenient method so far available. On the other hand, the apparently simple method of measuring insulin through its carbohydrate or glucose equivalent did not yield the definite results expected. A remarkable variation was found and considerable confusion arose during the earlier clinical tests. Williams (2) stated "No constancy has been found between the number of units of insulin administered and the amount of glucose which may be utilized," and Allen (3) concluded "that all rules based on the glucose value of the diet or any other principle yet known are widely fallacious." Even Wilder, Boothby and associates (4) who favored the adoption of a glucose standard for insulin, reported very divergent values, ranging from 0.9 to 3.1 grams per unit. Yet, on the average, it has been found that one clinical unit of insulin accounts for from 1.5 to 2 grams of carbohydrate and this value, although only approximate, has been of service in estimating the dosage to be given patients.

It was expected that more accurate information might be obtained by the use of dogs rendered totally diabetic by removal of the pancreas. The human patient has usually a certain amount of insulin from his own pancreas, the amount of which cannot be known and is probably variable, so that it is unlikely that the effect brought about by injected insulin

could be properly estimated. In the dog the only insulin available is that administered so that the uncertain factor of endogenous production is eliminated. The insulin which can be extracted from the tissues of depancreatized animals is apparently not in a form available for the metabolism of sugar.

The use of depancreatized dogs as test objects for insulin, while it has yielded certain information regarding the quantitative behavior of the hormone, has not furnished the result especially desired, namely, a simple and accurate method of assay. The reason is that the glucose equivalent of insulin, that is, the number of grams of glucose which can be metabolized under the influence of one unit of insulin, has been found to be subject to great variation. Some of the factors responsible for this variation have been recognized, and as reported in a previous communication (5) the most important of these depends on the fact that the amount of sugar metabolized per unit varies with the size of the dose, becoming smaller as the dose is increased. In this respect insulin behaves according to the logarithmic law, which Murray Lyon (6) has demonstrated for adrenalin and which is also true for other drugs. The glucose equivalent also varies with the amount of carbohydrate in the diet, becoming larger as the latter is increased. This is shown by the fact that when sugar is added to the diet of a diabetic animal which is receiving daily an amount of insulin which is insufficient to keep it sugar-free, not all of the added sugar is excreted.

The method used in determination of the glucose equivalent clinically is usually as follows: After the glucose tolerance of a severe diabetic has been carefully determined, the increased tolerance resulting from administration of a definite dose of insulin is noted. In some cases the diet is kept constant, in other cases it is increased when insulin is given. In either case the number of grams accounted for by each unit is calculated by dividing the extra amount of glucose metabolized by the number of units. The glucose equivalent in depancreatized dogs can be estimated somewhat more directly, for here the entire amount of insulin available is that administered. The glucose excretion is deducted from the total glucose intake (from carbohydrate and protein) and the difference, being the total glucose metabolized, is divided by the number of units. The conclusions published in the previous paper were based on figures obtained by the latter method of calculation. If the former method is used exactly the same relations between dose and effect hold, although the actual values for the glucose equivalents are lower as will be evident from a study of figure 1 and table 1.

In this figure are shown the results obtained by administration of varying doses of insulin (lot no. 211) to two dogs fed a constant diet consisting of 500 grams of fresh chopped beef freed from visible fat and 100 grams cane sugar. The total of grams of glucose metabolized each day is plotted

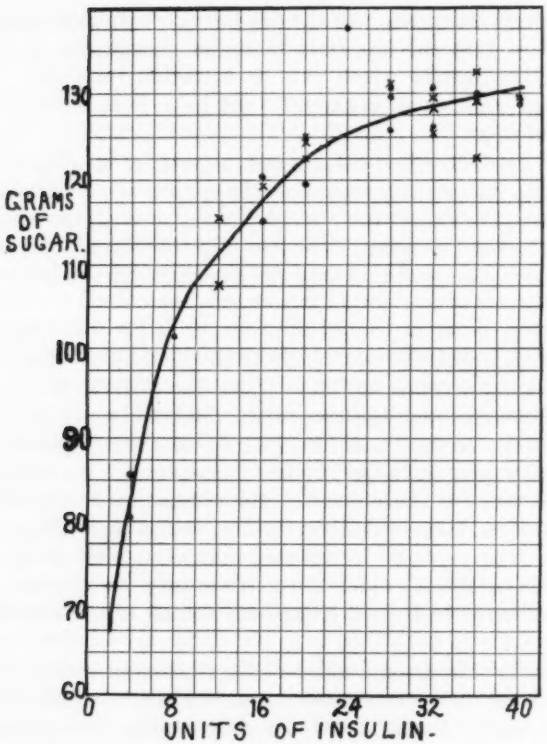


Fig. 1

TABLE 1

*Insulin 211. Dogs I and J*

| NUMBER OF UNITS | TOTAL GLUCOSE METABOLIZED | NUMBER OF GRAMS PER UNIT (GLUCOSE EQUIVALENT) | |
|---|---|---|---|
| | | 1 | 2 |
| 4 | 82.0 | 20.5 | |
| 8 | 100.0 | 12.5 | 4.5 |
| 12 | 111.5 | 9.3 | 3.7 |
| 16 | 117.5 | 7.3 | 3.0 |
| 20 | 121.5 | 6.1 | 2.5 |
| 24 | 125.0 | 5.2 | 2.2 |
| 28 | 127.0 | 4.5 | 1.9 |
| 32 | 128.5 | 4.0 | 1.7 |
| 36 | 130.0 | 3.6 | 1.5 |

1. Glucose equivalent obtained by dividing the total glucose metabolized by the number of units given.

2. Glucose equivalent obtained by dividing the extra sugar metabolized by the extra number of units given, the 82 grams metabolized with four units being taken as the basis.

against the number of units of insulin given in that day, the results of one dog being marked with dots, and the other with crosses. Although there are wide deviations, it is evident that the results fall along a curve which is steep where the dosage is small and flattens out where the dosage is large.

With 4 units of insulin, 82 grams of glucose (reading from curve) can be metabolized and with 8 units 100 grams, an increase of 18 grams for an addition of 4 units. The glucose equivalent would, therefore, be 4.5 according to the clinical method of calculation. The addition of 4 more units raises the metabolized glucose to 111.0, the glucose equivalent of this added amount being now only 2.8. The next addition of 4 units accounts for 6.5 grams, the next 4, for 4 grams, and so on, each increment having slightly less effect per unit than the previous amount. The curve flattens out where the large doses are given, so that there is less difference between the glucose equivalents. Here there is almost a linear relationship between dose and effect.

The low glucose equivalents obtained with the larger doses correspond with those obtained clinically, these, as a rule, being arrived at under corresponding conditions. It is evident that the administration of a small dose of insulin to a diabetic patient cannot have as high a glucose equivalent as the same dose given to a totally diabetic dog. In the former case the effect of the injected insulin is added to that of the endogenous insulin so that the total amount acting is relatively high and the glucose equivalent per unit correspondingly lower. The fact that the amount of sugar utilized per unit of insulin is approximately the same in diabetic dogs and human patients, when conditions are comparable, apparently indicates that the body weight has little influence on the power of insulin to enable the body to utilize carbohydrate. This conclusion is in accord with the clinical findings of Campbell (7) and Joslin (8), who have not found the insulin requirement to be affected to any appreciable degree by the size or weight of the patient. Still it is surprising that there should be so little difference in the results, with such differences in body weight as exist between these dogs of 7 or 8 kilograms and human patients, especially since the weight of the animal has apparently a decided influence in the rabbit assay method. Yet there is no inconsistency for even if exactly the same amount of sugar were removed from the blood by a unit of insulin in every case, the reduction in the percentage in the blood would, of course, vary with the total amount of sugar present, which is proportional to the size of the animal. There are, however, other factors not as yet clearly understood which even here are more responsible than the body weight for variation in the effect of insulin on different animals.

An attempt was made to learn what effect the nature of the food might have on the glucose equivalent, but the value of depancreatized dogs for such an experiment is limited by the defective digestion consequent on the

loss of the pancreatic juice. There has been clinical evidence that glucose derived from protein is metabolized with greater difficulty than that derived from carbohydrate. This appears to be the case but it has been difficult to make an accurate comparison in the experiments so far completed.

Even when all the conditions discussed above are controlled, as is the case of a dog fed the same diet and given the same amount of insulin, variations in the utilization of sugar shown by wide differences in the daily excretion of sugar still appear. Explanation for some of the differences can be found in technical errors, some of which are entirely unavoidable. Apart from the recognizable errors in incomplete collection of urine, by loss of a portion in the cage washings or otherwise, and in faulty administration of insulin by leak from syringe, or through the skin at the site of injection, there are the possible errors involved in absorption of the insulin and in absorption of the food from the bowel. It is probable that there may be considerable difference in the rate of absorption in the subcutaneous tissue in different parts of the body and injections given repeatedly in the same area are likely to cause changes which will interfere with absorption.

Excretion of insulin by the kidney has been shown to occur soon after injection, so that the rate of absorption of a dose may be very important. The absorption of the food from the bowel while fairly constant from day to day may vary sufficiently to cause a definite change in the glucose available for utilization. The variation is particularly probable in the protein assimilation. Yet, after consideration of all these factors, the explanation for the differences in excretion is still incomplete. Whatever may be the reason, it is obvious from inspection of the curve that an accurate assay of insulin cannot be made simply by finding out how much sugar is excreted and metabolized in a given instance, for the difference of 5 or 10 grams may mean a difference of 5 units or more when only 20 or 30 units are given altogether. Only by taking a very large number of observations and taking the average can anything approximating the true result be obtained. The practical value of the method for direct assay of insulin is therefore almost negligible.

In the light of these findings it is apparent that the clinical assay of insulin can be of little value, for on both theoretical and practical grounds the use of diabetic animals appears to offer much greater possibility of obtaining accurate and consistent results. If clinical assay is attempted, correct conclusions can only be drawn if a large number of individuals are tested over a considerable period, and if the nature of the diet and dosage are considered. Fortunately by means of assay by the selective rabbit method (9), satisfactory standardization of preparations of insulin for clinical use has been possible.

## CONCLUSIONS

The number of grams of glucose which can be metabolized in the body, under the influence of one unit, is a relative value, and not constant. It has been found to vary depending on the size of the dose and on the level of carbohydrate intake. In practice the former has less influence since the amount of insulin concerned is so large that the relationship between dose and effect becomes almost linear. The size or weight of the body has apparently no effect on the glucose equivalent.

The use of depancreatized dogs has not yielded a practicable method of assay, and the possibility of obtaining results of any value by clinical assay is doubtful.

In conclusion, the author wishes to express his appreciation of the assistance given by Dr. J. Markowitz and Mr. B. R. Dickson in performing some of the tests in the latter part of this investigation.

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